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THE VITAL CAPACITY OF THE SIAMESE

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Studies in recent years have indicated that race is a factor affecting vital capacity measurements. As no measurements have heretofore been made upon Siamese, the writer reports the following study made upon a hundred Siamese men. As is well known, age, height, weight, body surface, sex, posture, and state of health are all factors giving variety to vital capacity measurements (Myers, 1925). These factors have been held constant, so far as has been practicable, in making the measurements here reported.

MATERIAL AND MEASUREMENTS. The present measurements were made upon men of ages 21, 22, and 23 years, soldiers in the Siamese army. All were in good health at the time of measurement, as far as was obvious to ordinary observation of their appearance and behaviour. All had been given four hours of drill each day for six months or more before the measurements were made.

The vital capacity was measured in the standing posture. Three measurements were made upon each man and the highest reading was accepted. One hundred men were measured. The men were weighed dressed. All wore similar uniforms. Three of these uniforms were weighed and averaged and the mean was subtracted from the body weight of each man as measured. The difference was accepted as the net body weight. The body surface area was computed from the height and net weight with the aid of the Du Bois chart. The chart was graduated to read to hundredths of a square meter. The vital capacity per square meter of body surface was calculated. The original data are not presented, for lack of space, but those most essential are summarized in tables 1 and 3.

Analysis of data. All statistics are calculated from the original values without grouping. The distribution of the series of vital capacity values was tested for asymmetry and for flatness by calculation of the statistics γ_1 and γ_2 (Fisher, 1925). The first statistic (γ_1) has the value -0.0018

$\pm$ 0.1652, representing an asymmetry in which the mode of the series lies at a higher level than the mean; the second (γ_2) has the value -0.6144 ± 0.3304 corresponding to a distribution curve flatter and narrower at the base than the normal curve. If the true distribution of vital capacity measurements were normal, a degree of asymmetry as great as that found here would probably occur due to chance alone in 99 out of 100 series of measurements such as this; and a degree of flatness as great as this sample shows would occur in over 20 out of 100 such series. As there is not sufficient evidence, therefore, to make it probable that the variable departs from the normal distribution, we assume, pending collection of more data,

TABLE 1

Vital capacity frequency

Vital capacity, liters (grouped) (1)	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
Number of persons in group (2)	1	7	9	16	17	12	21	11	4	1	1

TABLE 2

Means and standard deviations

MEASUREMENT	MEAN OF 100 VALUES	PROBABLE ERROR OF THE MEAN	STANDARD DEVIATION OF 100	PROBABLE ERROR OF THE STANDARD
	(1)	(2)	(3)	(4)
Age (years)	21.5300			
Height (centimeters)	163.1770	$\pm$0.3936	5.8347	$\pm$0.2783
Weight (kilograms)	54.7740	$\pm$0.3474	5.1505	$\pm$0.2456
Surface area (sq. M.)	1.5833	$\pm$0.0063	0.0935	$\pm$0.0044
Vital capacity (liters)	2.9016	$\pm$0.0273	0.4047	$\pm$0.0194
Vital capacity (lit./sq. M.)	1.8232	$\pm$0.0143	0.2120	$\pm$0.0102

that vital capacity under the conditions of these measurements is a normally distributed variable.

If vital capacity under the conditions of these measurements is a normally distributed variable, and if the mean and standard deviation of an indefinitely large series ("true" mean and standard deviation) are correctly represented by their estimates from this series of measurements (table 2), about 99 per cent of all values measured upon such material should lie between the limits 1.8575 and 3.9425 liters. None of the 100 values in the present series lies outside these limits. The mean vital capacity of these one hundred soldiers is 2.90 ± 0.0273 liters. On the assumption that such means are normally distributed the probability is estimated to be about 99 per cent that the true mean vital capacity of such material lies between the limits 2.80 and 3.00 liters.

The vital capacity per square meter of body surface was calculated on the assumption that vital capacity is a simple linear function of surface area. Table 3 presents data from which the assumption can be tested.

If the variable is normally distributed and if the true mean and standard deviation of such material are correctly represented by their estimates from this series of measurements (table 2) about 99 per cent of all values of vital capacity per square meter of body surface measured upon such material should lie between the limits 1.2739 and 2.3661 liters per square meter of body surface. With the same probability the true mean should lie between 1.7656 and 1.8743 liters per square meter of body surface.

The coefficient of variation of vital capacity is 13.9 per cent; that of vital capacity per square meter of body surface is 11.6 per cent. There is a decrease in the coefficient of variation of approximately 2 per cent, amount-

TABLE 3

Vital capacity against surface area

Surface area, square meters (grouped) (1)	1.4	1.5	1.6	1.7	1.8	1.9
Number of persons in group (2)	3	42	32	17	5	1
Mean vital capacity for the group (3)	2.5433	2.6957	2.9228	3.2223	3.2840	3.5800

TABLE 4

Correlation coefficients

Weight and vital capacity	±0.4948	±0.0509
Height and vital capacity	±0.5441	±0.0475
Body surface area and vital capacity	±0.5864	±0.0443
Height and vital capacity, weight partialed out	±0.3733	±0.0580
Weight and vital capacity, height partialed out	±0.2770	±0.0623

ing to more than a 16 per cent reduction in the variability, when the factor body surface, as it affects vital capacity, is held constant. It would seem therefore that a standard table of vital capacity per square meter of body surface would permit more precise prediction of the result of measurement upon an individual person than would a table simply of vital capacity.

The degree of correlation of vital capacity with the other body measurements made is shown in table 4. The degree of correlation with body surface is the highest and is significantly greater than that with body weight. The fact that the coefficient of correlation of vital capacity with body surface is higher than with other measurements lends support to the practice of expressing vital capacity in terms of liters per square meter of body surface, until a higher correlation is discovered.

Discussion. The vital capacity of these men is lower than that reported for white persons in America. West (1920) reported the normal vital

capacity for the American white male to be 2.5 liters per square meter of body surface. This is nearly 0.7 liter per square meter higher than was found in the 100 Siamese soldiers. Race as apparently a factor affecting vital capacity was also demonstrated for negro children by Wilson and Edwards (1922), Smillie and Augustine (1926), and Roberts and Crabtree (1927). According to all observers the vital capacity is lower in the negro than in the white race. It has also been found to be lower in the Chinese (McCloy, 1927) and Filipinos (Nanagas, 1927).

To what extent these racial differences are hereditary and to what extent due to factors in the habits and environment of the people are questions that remain unanswered.

SUMMARY

1. Vital capacity measurements were made upon 100 Siamese soldiers. The mean vital capacity is 2.90 $\pm$ 0.0273 liters, or 1.82 $\pm$ 0.0143 liters per square meter of body surface.

2. The distribution of vital capacity measurements such as these is very probably symmetrical, and is assumed to be normal in type.

3. The vital capacity of these men is more highly correlated with body surface area than with height or weight, and seems to have a linear relation to body surface.

The writer expresses his sincere thanks to Dr. E. C. Albritton for suggestions regarding the analysis of data and for other helpful suggestions and criticisms.

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EFFECT OF CONFINEMENT ON THE GROWTH OF CHICKEN COMBS AND TESTES[1]

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The growth and health of chickens are dependent on inherited characteristics, quality and quantity of feed consumed, environment and freedom from disease. Recently, to control intestinal parasites, the use of wire-floored brooder houses and battery brooders has been introduced. This practice, while successful in the control of certain infestations, has raised serious problems for investigation. Our efforts have been directed toward ascertaining the conditions which would permit the growth of chickens indoors and in coops which limit their activities, that would approximate that of chickens raised under normal farm environment.

At the Kentucky Agricultural Experiment Station an experiment is in progress to determine the effect on the growing chick of confinement under laboratory conditions, as compared with normal farm conditions. With this end in view, 360 one-day-old chicks which came from a common parent stock were divided into three groups of 120 each and placed under the following conditions. Lot 1 was raised in colony brooder houses with free access to grass plots and direct sunlight. Lot 2 was kept for six weeks in a hot-water-heated battery brooder of the usual type, containing a series of five superimposed wire-floored pens, each measuring 36 × 36 inches, 9 inches high and so situated as to receive no direct sunlight. The chicks were then transferred to the colony brooder house used for lot 1. Lot 3 was raised for six months in the battery brooders.

At the age of 10 weeks, the cockerels in lots 1 and 2 were separated from the pullets and placed in a colony brooder house which was similar in every way to that used for the pullets, where they were kept until maturity. The cockerels and pullets in lot 3 were separated and transferred to unheated broiler battery pens, the compartments of which were 36 × 36 inches, 18 inches high.

The same ration was fed the three lots. This had been found experimentally to supply all the necessary food factors for the growing chicks.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

It consisted of wheat bran 25, wheat middlings 25, ground yellow corn 25, meat scrap (50 per cent protein) 12, dried buttermilk 10, salt 1 and steamed bone meal 5 parts. Enough of a commercial cod liver oil concentrate was added to make 0.125 per cent of the mash. The mash and a grain mixture consisting of equal parts of cracked yellow corn and wheat were fed ad libitum in hoppers.

The chicks in each lot were weighed and observed individually every two weeks and records were kept of the mortality in each lot and the food consumed.

The chicks in lot 1, raised in the colony brooder house, did not grow quite so rapidly during the first six weeks as the chicks in lots 2 and 3, which were battery-brooder started and battery-brooder raised respectively.

During the twenty-four weeks over which this experiment extended, there was not a marked difference in the weights of the chicks in the three lots, the chicks in lot 3, however, were heaviest until the eighteenth week.

The chicks raised in the colony brooder house and those transferred to the colony brooder house when six weeks old, appeared decidedly more vigorous than those raised in battery brooders until mature.

Near the tenth week it was observed that the combs and wattles of the cockerels in lot 3 that were battery brooder raised during the experiment were distinctly larger than those in the other two lots. In some instances the combs, because of their weight and size, lopped to one side. This condition became more marked as growth progressed, until at the end of the experiment the comb in practically every case covered one eye. In many instances this resulted in the loss of sight in the eye covered by the comb.

The photograph shows a representative twenty-four-weeks-old cockerel selected from lots 1, 2 and 3, respectively, reading from left to right. The cockerels in lot 1, as represented by the cockerel in the photograph, were vigorous, healthy individuals, as were those in lot 2. This showed that the first six weeks of indoor confinement had little effect, if any, on the cockerels in lot 2. In lot 3, as represented by the cockerel in the photograph, the feathering was ruffled and the combs and wattles were approximately twice as large as those of the cockerels in lots 1 and 2.

Rickets did not occur in any of the three lots. Approximately 5 per cent of the confined chickens had slipped tendons or perosis.

TABLE 1

Weights of the 24-weeks-old cockerels and the weights and three greatest dimensions of their combs and testes

BREEDING CAPACITY	NUMBER OF COCKEREL	WEIGHT OF COCKEREL	COMB				TESTES			
			Weight	Length	Height	Thickness	Weight	Length	Breadth	Thickness
Lot 1—colony raised										
		grams	*grams*	*cm.*	*cm.*	*cm.*	*grams*	*cm.*	*cm.*	*cm.*
Good	5021	2216	34	12.3	6.7	1.2	8.3	4.1	2.0	1.5
	5313	2030	39	13.2	6.6	1.8	6.0	3.9	1.7	1.4
	5079	2132	42	13.2	6.9	1.9	5.9	3.8	2.1	1.6
	Average	2126	38.3	12.9	6.7	1.6	6.7	3.9	1.9	1.5
Poor	5024	1710	18	11.0	4.9	1.2	3.7	3.1	1.7	1.5
	5289	1951	25	11.2	6.0	1.2	2.8	2.8	1.5	1.5
	5173	1860	22	10.2	5.1	1.5	3.3	2.7	1.6	1.6
	Average	1840	21.7	10.8	5.3	1.3	3.3	2.9	1.6	1.5
Lot 2—Battery started										
Good	5073	1950	17	9.2	5.5	2.0	6.9	3.8	2.0	1.3
	5352	2425	40	12.7	6.3	1.5	4.1	3.8	1.5	1.2
	5138	1895	31	10.9	6.0	1.4	5.1	3.6	1.7	1.4
	Average	2090	29	10.9	5.9	1.6	5.4	3.7	1.7	1.3
Poor	5115	2480	48	13.5	8.0	1.8	5.0	3.5	1.5	1.2
	5176	1803	20	12.3	6.4	1.6	4.1	3.2	2.1	1.5
	5265	2015	32	11.9	7.3	1.5	3.3	2.9	1.3	1.3
	Average	2096	33	12.6	7.2	1.6	4.1	3.2	1.6	1.3
Lot 3—Battery raised										
Good	5160	1580	70	15.2	9.0	2.2	1.2	2.2	1.0	0.7
	5270	1960	104	16.6	10.9	1.4	3.1	3.1	1.8	1.4
	5316	1411	79	14.7	9.0	2.1	3.5	3.0	1.6	1.3
	Average	1650	84	15.5	9.6	1.9	2.6	2.8	1.5	1.1
Poor	5114	1370	62	15.2	9.0	1.5	2.5	2.9	1.5	1.2
	5141	1640	89	16.7	8.7	2.1	4.2	3.0	2.0	1.5
	5375	1256	69	16.0	9.5	1.2	0.5	1.4	0.9	0.5
	Average	1422	73	15.9	9.1	1.6	2.4	2.4	1.5	1.1

At the end of the twenty-fourth week, three cockerels which were determined by external examination to be in good breeding conditions and three

cockerels which by similar examination were determined to be in poor breeding condition were selected from each lot, weighed individually and killed. The combs were cut off, weighed and measurements made of their greatest length, height and thickness. The body was then opened and the testes removed, weighed and measurements made of their greatest length, breadth and thickness. These data are stated in table 1.

Even though the cockerels selected from lot 3 were smaller than those from lots 1 and 2, the average weights and sizes of their combs were more than twice as large. The average weights of the testes of the cockerels from lot 1, raised in colony brooders, were much larger than those of the cockerels from lot 3, raised in battery brooders. The testes of the cockerels from lot 3, besides being smaller, were found to be less moist when cut than those from the other two groups. While it was impossible to estimate accurately the comparative number of spermatozoa in the semen from the three sets of cockerels, it was observed that there was a decidedly smaller number in that obtained from the seminal vesicles of the cockerels in lot 3.

The fertilizing capacity of the cockerels in lot 3 was found to be low as compared with the cockerels in lots 1 and 2 when mated with hens which had previously produced hatchable eggs. This may have been partly due to the smaller number of spermatozoa and to the enlarged combs and partial blindness which unbalanced the cockerels and prevented successful copulation.

The cause of the production of large combs and small testes in cockerels raised in confinement and in the absence of direct sunlight is unknown. It seems possible that the enlarged combs were associated with the exclusion of direct sunlight, the cockerels elaborating combs with larger surfaces to compensate for the decreased quantity of solar radiation received. The development of smaller testes which in two instances were rudimentary may have been due also to the decreased quantity of solar radiation received. It is a well known fact that close confinement is not beneficial for breeding males and that the lack of exercise usually leaves the male somewhat heavier than usual and in poorer breeding ability.

Womack, Koch, Domm and Juhn (1931) have shown that light retards or lowers the comb growth response in Brown Leghorn capons to a given dose of testicular hormone and discuss unpublished data of Dr. L. V. Domm concerning the seasonal variations in the sizes of combs of normal cocks and poulardes which are possibly influenced by light and exercise.

Search of the literature has failed to reveal any previous reference to these phenomena in White Leghorn cockerels. This may be because confinement has been practiced for only a short time or because the males usually are sold as broilers at an early age. Further work along this line has been undertaken to find the effect on the testes of the cockerels when the combs are removed at an early age.

These results show that cockerels raised in confinement in battery brooders, from which direct sunlight was excluded, developed abnormally large combs and smaller testes than those raised in colony brooders, with access to a grass range and receiving direct sunlight.

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FACTORS INFLUENCING THE PASSAGE OF LIQUIDS FROM THE STOMACH INTO THE INTESTINE[1]

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The subject under consideration is not new. In fact, the number of investigators associated with it is so large that in our paper only a brief mention of their accomplishments is possible.

The first and most celebrated of these was William Beaumont (1). The vast number of his careful observations upon Alexis St. Martin are yet regarded as worthy of emulation. Important advances in gastro-intestinal physiology were made by Hirsch (2), Marbaix (3), von Mering (4) and Moritz (5) among physicians and Pavlov (6) and Cannon (7) among physiologists. With the name of Marbaix (3) is associated the phrase "test portion." As the stomach is filling, a small amount of the contents passes into the intestine. If it is of the proper reaction and state of digestion the remainder is evacuated promptly. If unfavorable conditions exist, however, the intestine defends itself against invasion either by closure of the pylorus or by anti-peristaltic movements. This investigator was the first to emphasize the importance of the condition of the intestine in gastric evacuation. Especially abundant and valuable material in this field was presented by Pavlov (6) and his school. A study of liquid substances occupied most of their time. Pavlov and Serdiukoff (8) reported that acids are retained in the stomach for a long time. Neutral solutions pass into the intestine very freely, depending upon the evacuation time of that organ. They further found that alkaline solutions do not delay gastric evacuation but on the contrary accelerate it, and pass into the duodenum even more rapidly than neutral liquids such as water.

Pavlov and his pupil Lintvareff (9) established the fact that liquefied fat passes readily into the intestine but is promptly regurgitated into the stomach. Such exchange between the intestine and stomach may occur many times, depending upon the quantity and composition of the fat and the condition of the intestine.

In passing, we must mention the work of one of us (Boldyreff, 10) in 1911

[1] Reported April 28, 1932 at Annual Meeting of American Physiological Society in Philadelphia.

which showed that not only acids and fats but all strong irritants, even alkalies, are retained unusually long in the stomach.

Apperly and Crabtree (11) have recently reported that "the emptying time of the stomach is determined by the pH of the blood."

In such a short résumé, one is obliged to enumerate only the most important contributions to the knowledge of the subject. It is to be regretted that space forbids a further discussion of them.

EXPERIMENTAL PROCEDURES. Of the five dogs used in our observations, four had gastric fistulae. The other had a fistula tube in the duodenum. Dog 27, male, weight 20,700 grams, age 7 years; dog 120, female, weight 12,500 grams, age 7 years, intestinal fistula; dog 145, female, weight 13,000 grams, age 1 year; dog 146, male, weight 13,000 grams, age 1 year, suffered from duodenitis and pancreatitis as confirmed by autopsy; dog 150, male, weight 13,000 grams, age 1 year.

The liquids studied were introduced into the stomach through the fistula tube in 200 cc. quantities. Except in experiments on the influence of temperature, they were warmed to 40°C. before being used. At the end of a twenty minute interval the remaining liquid was removed from the stomach. The results obtained were tabulated for comparison and contrast.

In studying the passage of acids from the stomach, a slightly different technique was used. Two hundred cubic centimeters of acid of known strength were introduced into the stomach. At the end of twenty minutes the residue was measured and reintroduced, a small portion being reserved for titration. This procedure was repeated at intervals of twenty minutes until the stomach was practically empty. Thus the relation between gastric evacuation and neutralization was studied.

The fluoroscope was used in observing the action of the pylorus. An opaque mixture of slightly alkaline (0.5 per cent MgO) starch (2 per cent) and barium sulphate was introduced into the stomach either by gastric tube or through the fistula. Roentgenograms were taken.

The action of irritants can be best divided into two groups, chemical and mechanical. It should be mentioned now that no solutions were used which were too strong to be taken by mouth. All of the liquids were tested on man before being introduced into the dog's stomach. Pepper (0.3 per cent), ginger (1 per cent), currie (2 per cent) and mustard infusions (1 and 3 per cent) and alcohol in solutions of 1 to 15 per cent belong to the class of chemical irritants. In order to avoid any mechanical action these substances were filtered. Nevertheless their inhibitory action was marked. Mustard and pepper prolong the time required to empty the stomach to twice or three times that seen with plain tap water. Little delay is noted when alcohol is administered in dilute concentrations. However, in strengths of 5 to 15 per cent it is strongly inhibitory. The delay is similar

to that observed with mustard and pepper. The action of psyllium seed and bran as a mechanical irritant was studied with considerable interest. Although our number of observations is limited, they appear to confirm the statement that gastric evacuation is hastened by their presence.

Acids have long been known to be slow in leaving the stomach. The inhibitory action appears to be in direct proportion to the strength of the solution; weak lactic acid (less than 0.1 per cent) and HCl (0.05 per cent and less) are evacuated as if they were tap water. Many observations on lactic acid of various higher concentrations (0.1–0.6 per cent) show that they delay evacuation. Such an action is more pronounced with hydro-

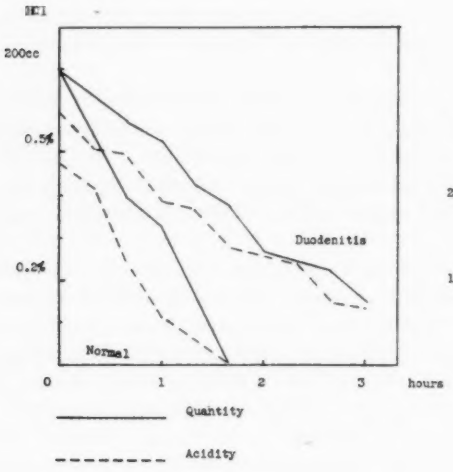

Fig. 1

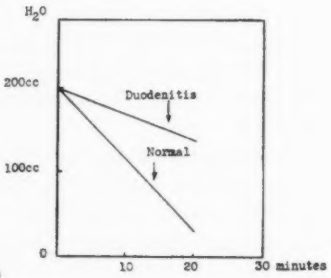

Fig. 2

Fig. 1. Gastric evacuation of HCl
Fig. 2. Gastric evacuation of water

chloric acid; in a normal dog, it requires one hour to one hour and a half to empty the stomach of 200 cc. gastric juice of 0.5 per cent HCl. A similar amount of water passes into the duodenum in approximately twenty or thirty minutes.

One of the most fruitful portions of our research was that which concerned alkalies. Contrary to the supposition that alkaline solutions leave the stomach more quickly than neutral ones, it was observed that their action was markedly inhibitory. Excepting with very weak solutions (0.5–1 per cent $NaHCO_3$) this was observed in every case. Substances used were: $NaHCO_3$ 0.1–6.4 per cent, unfiltered calcined magnesium 1–2 per cent, filtered calcined magnesium 2 per cent, filtered lime-water 0.6

TABLE 1

Effect of gastritis on gastric evacuation

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes at 40°C.

Tap water*	86	50	58	40	60	72	58	78
NaCl, 0.9 per cent*	48	83	84					
NaCl, 0.3 per cent*	103							
Distilled water*	50							
$MgSO_4$, 2 per cent*	93							
Na_2SO_4, 2 per cent*	90							
Tap water control	30	30	24	19				

* Normal figure for each of these is 20–30 cc.

Remark: All the above figures are obtained on a dog suffering from gastritis, except the last row, tap water control, which refers to a normal animal.

Experiments from October 5 to December 31, 1931.

TABLE 2

Effect of duodenitis on gastric evacuation

Dog N 146. Two hundred cubic centimeters of liquids introduced into the stomach at 40°C.

Tap water, 20 minutes	97	92	125	66	134	36
Gastric juice, 0.60 per cent HCl, 20 minutes	184					
Lactic acid, 0.067 per cent, 20 minutes	78					
Lactic acid, 0.11 per cent, 10 minutes,	70					
15 minutes	43					
Lactic acid, 0.22 per cent, 10 minutes	82					
Lactic acid, 0.90 per cent, 20 minutes	148					
Tap water control, 20 minutes	30	30	24	26	30	

Remark: All these figures, except the last line, refer to a diseased animal.

Experiments from November 4 to December 4, 1931.

TABLE 3

Effect of irritants on gastric evacuation

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes at 40°C.

1 per cent mustard infusion	135				
3 per cent mustard infusion	87				
0.33 per cent pepper infusion	70				
2 per cent currie infusion	81				
5 per cent very old horse radish infusion	110				
1 per cent alcohol	46				
2 per cent alcohol	30	30			
4 per cent alcohol	22				
5 per cent alcohol	74				
10 per cent alcohol	68	104			
15 per cent alcohol	106				
Tap water control	30	30	30	19	21

Experiments from July 26, 1931, to May 26, 1932.

per cent, filtered MgO 1 per cent and NH_4OH 0.5–1 per cent–1.5 per cent.

The action of some neutral salts was found to be similar in many respects to that of alkalies. $NaCl_2$ 2 per cent, $MgSO_4$ 2 per cent and Na_2SO_4 2 per

TABLE 4

Evacuation of alkalies

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes.

$NaHCO_3$, 6.4 per cent, 20°C.	127					
$NaHCO_3$, 3 per cent, 20°C.	147					
$NaHCO_3$, 2 per cent, 20°C.	100	88				
$NaHCO_3$, 1 per cent, 20°C.	8	34				
$NaHCO_3$, 0.5 per cent, 20°C.	30					
Filtered calcined Mg, 2 per cent, 40°C.	38	54				
Unfiltered calcined Mg, 2 per cent, 40°C.	68					
NH_4OH, 0.5 per cent, 40°C.	47					
NH_4OH, 1 per cent, 40°C.	42	64				
Filtered lime water, 0.6 per cent, 40°C.	117					
Filtered MgO, 1 per cent, 40°C.	51					
Tap water control, 40°C.	30	24	30	26	30	30

Experiments from October 1, 1931, to January 5, 1932.

TABLE 5

Evacuation of acids

Two hundred cubic centimeters of liquids introduced into the stomach at 40°C.

HCl, 0.60 per cent, 20 minutes	196						
Gastric juice, 0.51 per cent HCl, 20 minutes	139						
HCl, 20°C., 0.2 per cent, 20 minutes	125						
HCl, 0.11 per cent, 20 minutes	100						
HCl, 0.05 per cent, 20 minutes	30	22					
Lactic acid, 0.63 per cent, 20 minutes	126						
Lactic acid, 0.22 per cent, 15 minutes	78						
Lactic acid, 0.125 per cent, 20 minutes	44						
Lactic acid, 0.1 per cent, 20 minutes	40						
Lactic acid, 0.07 per cent, 15 minutes	40						
Tap water control, 20 minutes	30	28	30	30	24	26	30

Experiments from October 1, 1931, to May 21, 1932.

cent were notably inhibitory. Observations on mineral oil showed it to leave the stomach in approximately the same time as does tap water.

Quinine (0.5 per cent) and peptone (10 and 20 per cent) caused inhibition to gastric evacuation. The action of the former was mild, the latter severe. With 20 per cent peptone only 18 cc. of solution left the stomach in the twenty minute interval. Starch in 2, 3, 4 and 6 per cent solutions appeared to have little action.

TABLE 6

Miscellaneous substances

Two hundred cubic centimeter portions introduced into the stomach for 20 minutes.

NaCl, 4 per cent, 20°C	200			
NaCl, 2 per cent, 20°C	100			
NaCl, 0.9 per cent, 20°C	48*	83*	84*	18
NaCl, 0.3 per cent, 20°C	103*	24		
Distilled water, 40°C	50*	20	21	20
$MgSO_4$, 2 per cent, 40°C	98			
Na_2SO_4, 2 per cent, 40°C	69*	148*	20	
Na_2SO_4, 4 per cent, 40°C	48			
Na_2SO_4, 6 per cent, 40°C	178			
Mineral oil, 45°C	22			
Starch, 2 per cent, 40°C	38			
Starch, 3 per cent, 40°C	44			
Starch, 4 per cent, 40°C	40			
Starch, 6 per cent, 40°C	40			
Peptone, 10 per cent, 40°C	60			
Peptone, 20 per cent, 40°C	182			
Egg white	38			
Quinine, 0.5 per cent, 40°C	37			
Tap water control, 40°C	30	30	30	

* These figures are to be explained by the presence of an acute gastritis, as was found later.

Experiments from October 5, 1931, to May, 1932.

TABLE 7

Effect of temperature on evacuation of tap water

Two hundred cubic centimeters of water introduced into the stomach for 20 minutes.

2°C	40							
3°C	51							
4°C	24	45						
6°C	36							
8°C	72*							
10°C	27							
12°C	34							
15°C	40							
20°C., room temperature	30							
40°C., body temperature	30	28	30	30	24	26	30	30
45°C	70*							
46°C	56							
48°C	50							

* Large figures are to be explained by the presence of an acute gastritis, as was proven later.

Experiments from October 1, 1931, to March 31, 1932.

It was incidentally found that sometimes apparently normal dogs are suffering from acute gastritis. In such cases the evacuation of different substances was greatly retarded. (See tables 6 and 7.) Even tap and distilled water was retained in the stomach for a considerable period of time.

On one dog, used for experimentation, the results obtained were markedly different from those observed on other subjects. All solutions, even tap water, were retained in the stomach much longer than had been considered normal. Post-mortem examination of this animal revealed the presence of both duodenitis and pancreatitis. (See table 2.)

A similar observation was made on a dog which chewed up his fistular tube and swallowed it. An acute duodenitis ensued and the same phenomena were exhibited as observed in the dog before mentioned. The end results of this latter case were more gratifying as the dog recovered.

In view of the fact that a great deal of importance has been attached to the temperature of liquids entering the stomach, some observations were undertaken to determine the influence of water at varying temperatures on the emptying time of this organ. A great deal of care was exercised in getting a true control group of experiments. A body temperature of 40°C. was chosen as the optimum for the subjects. Tap water[2] was cooled to 2°, 3°, 4°, 6°, 8°, 10°, 12°, 15° and 20°. At these temperatures there was no case in which evacuation was markedly inhibited. The residues ranged from 24 to 51 cc. as compared with the 30 cc. standard at 40°C. A similar result was noted with temperatures of 45°, 46°, 48° and 50°.

Discussion. The rapidity with which the stomach empties itself of liquids appears to depend upon several factors. The first and most important of these concerns gastric acidity. Acid secretion may result from psychic stimuli or from the presence of specific secretagogues in the viscus. Such excitants as the sight and smell of food can be eliminated but it is more difficult to keep the dogs from misinterpreting certain activities, particularly sounds, in their environment such as preparations for feeding, etc.

There are many substances which excite a definite flow of gastric juice. Among the more familiar ones are: water (tap and distilled), NaCl, alcohol and peptone. Several others which were unknown are: ammonia, MgO (1.5–0.125 per cent), $MgSO_4$ (2 per cent), and quinine (0.5 per cent). Of these peptone, alcohol and ammonia are the strongest. Doubtless this action sometimes plays a very important rôle in inhibiting gastric evacuation. A solution of peptone had the longest emptying time and was the most active in the production of gastric juice.

[2] This water was analysed and the following figures were obtained: total solids 0.0372, organic matter 0.0127, SiO_2 0.0023, FeO 0.0003, Ca 0.0097, Mg 0.0021, Cl_2 0.0013, SO_4 0.0020. Parts per 100.

The action of some neutral salts as gastric stimulants is probably the underlying factor in their slow passage into the duodenum. Logically, therefore, the weaker the solution the more nearly it approaches the evacuation time of tap water. As mentioned in the tables, very weak acids and alkalies and some neutral salts do exhibit this property.

Since the discovery of the phenomenon of duodenal regurgitation by one of us (W. N. B.) a great deal of interest has been manifested in regard to its rôle in the regulation of gastric acidity. In our observations upon the evacuation of acids we noticed an interesting phenomenon. After the acid had been in the stomach for twenty minutes there was a marked decrease in acidity but little change in chloride content and volume. In one case there was actually more liquid present than was introduced through the fistular tube. The color was not infrequently yellow and flakes of mucus were also observed. Determinations of chloride content showed that while the acidity decreased very markedly, the fall in chlorides was not parallel. For example: In one hour the acidity fell from 0.51 per cent HCl to 0.20 per cent while the chlorides diminished from 0.53 per cent to 0.46 per cent (experiment of April 12, 1932). Such observations help us to understand more fully the delay in normal gastric emptying and that in the dog suffering from duodenitis and pancreatitis.

CONCLUSIONS

1. Gastritis and duodenitis inhibit gastric evacuation markedly. Even tap water is delayed two or three times the normal limits.
2. No other liquid leaves the stomach more rapidly than water.
3. Strong alkalies inhibit the emptying of the stomach as much as do acids.
4. Weak alkalies leave the stomach with the same rapidity as weak acids.
5. Gastric emptying is prolonged by alcohol, mustard, pepper, etc., some bitter salts and peptone.
6. Temperatures from 2° to 50°C. have very little effect upon the emptying time of the stomach.
7. Duodenal regurgitation is the most important factor in the neutralization of gastric acidity.

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THE NUTRITIVE PROPERTIES OF THE "CROP-MILK" OF PIGEONS[1]

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In 1786 John Hunter described the material formed in the crops of breeding pigeons for the nourishment of their young, as follows:

> The young pigeon, like the young quadruped, till it is capable of digesting the common food of its kind, is fed with a substance secreted for that purpose by the parent animal; not, as in the Mammalia, by the female alone, but also by the male, which, perhaps furnishes this nutriment in a degree still more abundant. . . . .
>
> During incubation the coats of the crop in the pigeon are gradually enlarged and thickened, like what happens to the udder of females of the class Mammalia in the term of uterine gestation. On comparing the state of the crop when the bird is not sitting, with its appearance during incubation, the difference is very remarkable. In the first case it is thin and membranous; but by the time the young are about to be hatched, the whole, except what lies on the trachea, becomes thicker, and takes on a glandular appearance, having its internal surface very irregular. It is likewise evidently more vascular than in its former state, that it may convey a quantity of blood sufficient for the secretion of the substance which is to nourish the young brood for some days after they are hatched.
>
> Whatever may be the consistence of this substance when just secreted, it most probably very soon coagulates into a granulated white curd, for in such form I have always found it in the crop; and if an old pigeon is killed just as the young ones are hatching, the crop will be found as above described, and in its cavity pieces of white curd, mixed with some of the common food of the pigeon, such as barley, beans, etc. If we allow either of the parents to feed the brood, the crop of the young pigeons when examined will be discovered to contain the same kind of curdled substance as that of the old ones, which passes from thence into the stomach, where it is to be digested.
>
> The young pigeon is fed for a little time with this substance only, as about the third day some of the common food is found mingled with it: as the pigeon grows older the proportion of common food is increased; so that by the time it is seven, eight, or nine days old, the secretion of the curd ceases in the old ones, and of course no more will be found in the crop of the young. It is a curious fact that the parent pigeon has at first a power to throw up this curd without any mixture of common food, although afterwards both are thrown up according to the proportion required for the young ones.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

Claude Bernard (1859) described the phenomenon at greater length and gave the details of an anatomical study of the crop of the pigeon. He found the so-called "crop-milk," or "pigeon-milk," to be composed of desquamated epithelial cells of the mucosa of the crop which clump together to form small masses having the appearance of milk curd. An analysis of the material showed protein and salts 23 per cent, fat 10 per cent, and water 67 per cent; sugar was not found.

A number of studies of the production of crop-milk, and of the histology of the tissues involved, have appeared since Claude Bernard's paper. Among these the work of Phisalix (1890) may be mentioned. Carr and James (1931) and Beams and Meyer (1931) are the most recent workers in this field.

Phisalix, by section of the pneumogastric nerve, had, in one experiment, prevented the proliferation of the mucosa of the crop at the usual time. Recently, however, Riddle and Braucher (1931) have brought about the regular sequence of events, even after section of the nerves, by injection of anterior pituitary extract, and have thereby demonstrated the hormonal control of the phenomenon.

Although considerable study has been devoted to the physiological and histological aspects of the production of crop-milk, little attention appears to have been paid to the somewhat extraordinary nutritive properties of this unique food material. Riddle (1928) has indicated that squabs increase in weight relatively faster than do the young of any other animal for which data are available. This rapidity of growth aroused our interest in crop-milk as a source of energy and of accessory food factors. We have accordingly studied the concentration of vitamins A and B (B + G) in samples supplied to us in daily shipments through the courtesy of Doctors Riddle and Bates of the Station for Experimental Evolution, Carnegie Institution of Washington, at Cold Spring Harbor, Long Island. The specimens were necessarily derived from different birds; they were obtained, in fact, not from the adults, but from the young soon after the crop-milk had been received from the parents. It was found by Doctors Bates that the thin crop wall of the young bird could be slit, the crop-milk removed, and the wound sutured; furthermore that this process could be repeated several times at daily intervals on the same squab without serious injury.

The diet of the adult pigeons consisted of wheat, yellow and kafir maize, and hempseed, together with suitable grits. A calculation of the essential nutrients in this diet indicated the composition: protein (crude) 14.4 per cent, nitrogen-free carbohydrate 62.9 per cent, fat 8.9 per cent, crude fiber 47 per cent, ash 2.2 per cent, water 10.3 per cent; kafir maize and wheat are sources of vitamin B, yellow maize of vitamin A.

The composition of the crop-milk was found, by analysis of pooled specimens, to be: protein (N × 6.25) 18.8 per cent, fat 12.7 per cent, ash

1.6 per cent, water 64.3 per cent. These data are in close agreement with the analysis quoted by Claude Bernard.

At hatching the squab already has some water and a small supply of food in its gut in the form of unabsorbed but modified residual egg yolk. It therefore seemed advisable also to assay the yolk of pigeon eggs for vitamin A, especially as this substance is known to be concentrated in the yolks of the eggs of other species. The eggs of pigeons were therefore also obtained from the laboratory at Cold Spring Harbor; these were heated in water to coagulate the yolks before being shipped.

The ether-soluble portion of the crop-milk was obtained by dehydrating a weighed sample with three successive portions of cold absolute alcohol. The residue was then extracted continuously for 48 hours with hot absolute alcohol and subsequently for about 4 hours with ether. The residual solid was dried in a desiccator, and finally at 90° in an oven in the presence of carbon dioxide, before being weighed. The alcoholic solutions from the cold and hot extractions were combined, concentrated *in vacuo* and freed

TABLE 1

SAMPLE	AMOUNT OF CROP-MILK	SOLID RESIDUE	LIPIDS	
	grams	*per cent*	*grams*	*per cent*
1	18.0	14.3	1.696*	9.4
2	30.0	15.4	2.697	9.0
3	46.5	18.6	5.936	12.7

* Lipids from cold alcohol extraction, iodine number 106.2, 106.5. Lipids from hot alcohol extraction, iodine number 114.0, 115.0.

from the last portions of alcohol in a vacuum desiccator at 5 mm. pressure. The lipids so obtained were taken up in absolute ether, the solution was filtered, and the ether evaporated. The lipids were then dried under a stream of carbon dioxide at 90°, weighed, and stored in a refrigerator in small vials under carbon dioxide. The hot and cold alcohol extracts from the first large sample of crop-milk received were worked up separately, and the iodine numbers of the lipids from each were determined by the Hanus method. The results are presented in table 1.

FEEDING EXPERIMENTS. Owing to the limited supply of crop-milk available relatively few animal experiments could be conducted; individual variations have therefore played a larger rôle in this investigation than is ordinarily desirable.

Vitamin A tests of crop-milk and pigeon egg yolk. Twenty male albino rats, weighing 40 to 50 grams at weaning, were fed on a diet, deficient in vitamin A, of the following composition: casein (extracted) 18 per cent, starch 63 per cent, lard 15 per cent, and salt mixture (Osborne and Mendel,

TABLE 2

Results of vitamin A assay of crop-milk and pigeon egg yolk

RAT NUMBER	SUPPLEMENT	AMOUNT OF SUPPLEMENT	INITIAL		DURATION OF EXPERIMENT	FINAL	
			Body weight	Ophthalmic condition		Body weight	Ophthalmic condition
		grams	*grams*		*days*	*grams*	
C3910	Crop-milk	0.5	97	Severe	8	77	Severe
C3914	Crop-milk	0.5	96	Severe	12	86	Severe
C3916	Crop-milk	0.5	87	Severe	12	85	Severe
C4033	Crop-milk	1.0	56	Severe	26	103	One eye cured, other nearly so
C4032	Crop-milk	2.0	61	Severe	5	68	Severe
		4.0	68	Severe	10	109	Cured (8th day)
C3900	Ether-soluble fraction of crop-milk	0.1	127	Severe	12	126	Severe
C3909	Ether-soluble fraction of crop-milk	0.1	112	Severe	8	121	Severe
C3913	Ether-soluble fraction of crop-milk	0.1	110	Severe	8	105	Severe
C3918	Ether-soluble fraction of crop-milk	0.1	92	Severe	8	85	Severe
C4043	Pigeon egg yolk	0.1	100	Severe	14	115	Cured
C4044	Pigeon egg yolk	0.1	124	Severe	8	132	Severe
		0.2	132	Severe	14	146	Cured
C4045	Pigeon egg yolk	0.1	104	Severe	22	119	Severe
		0.5	119	Severe	13	143	Improved
C4054	Pigeon egg yolk	0.1	109	Severe	8	110	Severe
		0.2	110	Severe	12	120	Severe
		0.5	120	Severe	6	132	Cured
C4060	Whole pigeon egg	3.0	85	Severe	1		
	Pigeon egg yolk	1.0			1		
	Pigeon egg yolk	0.5			3		
	Whole pigeon egg	0.5			2	96	Cured (6th day)
		drops					
C3917	Cod liver oil	1	77	Severe	13	114	Cured (6th day)
C4046	Cod liver oil	1	119	Severe	6	133	Cured (6th day)
C4055	Cod liver oil	1	91	Severe	9	115	Cured (6th day)
C3899	Peanut oil	1	94	Severe	15	68	Severe
C3912	Peanut oil	1	94	Severe	7	72	Severe
C4028	Peanut oil	1	61	Severe	25	52	Severe

At the termination of the experiment 10 drops of cod liver oil were added to the diets of rats C3914, C3916, C3909, C3913, C3918, and C4045. This resulted in a cure of the xerophthalmic condition.

1919) 4 per cent, plus 1 drop daily of maize oil (Mazola) that contained 0.001 mgm. of irradiated ergosterol, and 0.2 gram daily of dried brewery yeast. At the appearance of the usual symptoms of vitamin A deficiency—xerophthalmia and loss in weight—16 to 40 days later, the animals were

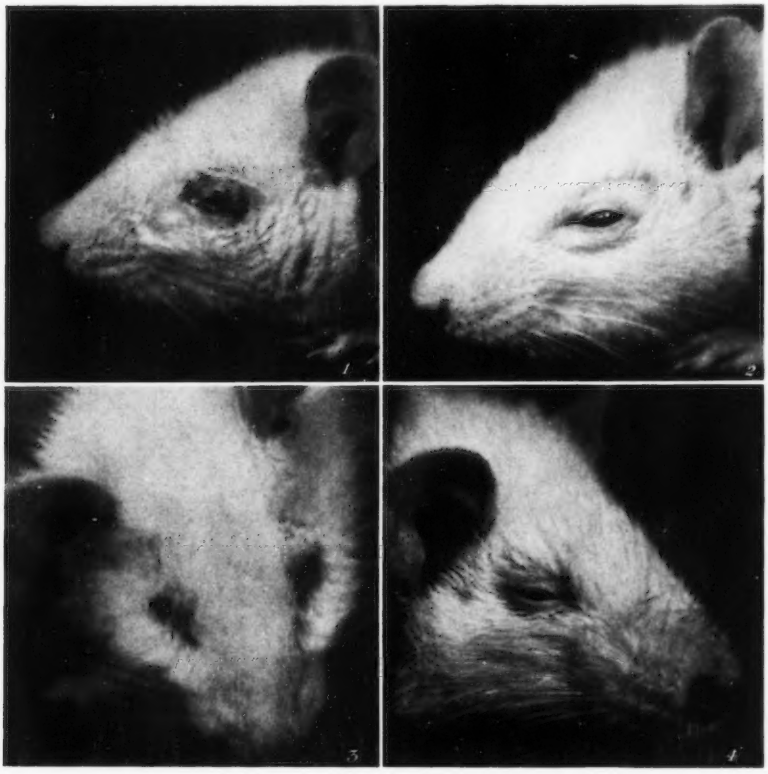

Fig. A. *1*. Rat C4033, the degree of xerophthalmia obtaining after the administration of 1 gram of crop-milk for 18 days. *2*. Rat C4032, a cure of xerophthalmia by 4 grams of crop-milk daily. *3*. Rat C4060, the pathological condition of the eyes before the basal diet was supplemented with pigeon egg yolk. *4*. Rat C4060, the improvement obtained in 6 days by feeding egg yolk.

divided into groups and the diet was supplemented as is shown in table 2. The crop-milk and pigeon egg were weighed to the nearest centigram and the ether-soluble fraction to the nearest milligram.

The results of these feeding experiments are presented in detail in table 2. They show, with respect to *crop-milk*, that a daily supplementary intake of

0.5 gram was inadequate to insure maintenance of body weight or relief of xerophthalmia; better results were secured with a daily dose of 1 gram; and an intake of 4 grams per day produced a prompt cure.

The *ether-soluble fraction*, when administered in daily quantities of 0.1 gram, or the equivalent of 1 gram of fresh crop-milk, prevented serious declines in weight but had no other apparent beneficial effect; owing to the scarcity of material it was not possible to increase the dosage.

TABLE 3

Results of vitamin B (B + G) assay of crop-milk

RAT NUMBER	SUPPLEMENT	AMOUNT OF SUPPLEMENT	GAIN IN BODY WEIGHT	PERIOD OF TEST	GAIN PER DAY
		grams	*grams*	*days*	*grams*
C4149	Crop-milk	0.5	−6	14	−0.4
C4161	Crop-milk	0.5	+5	14	+0.4
C4162	Crop-milk	0.5	+1	13	+0.1
					Av. +0.1
C4191	Crop-milk	1.0	+30	18	+1.7
C4228	Crop-milk	1.0	+6	21	+0.3
C4229	Crop-milk	1.0	+12	21	+0.6
					Av. +0.9
C4230	Crop-milk	3.0	+17	15	+1.1
C4150	Yeast	0.1	+38	20	+1.9
C4166	Yeast	0.1	+26	18	+1.4
					Av. +1.7
C4163	None		−3	25	−0.1
C4187	None		−9	27	−0.3
C4297	None		−1	27	−0.0
					Av. −0.1

At the termination of the experiment 0.2 gram of dried brewery yeast was added to the diets of rats C4149, C4161, C4162, C4228, C4229, and C4230. This brought about a normal rate of growth.

Pigeon egg yolk produced occasional favorable responses with daily intakes of 0.1 to 0.2 gram. Satisfactory cures were brought about by the ingestion of 0.5 gram a day.

"Positive control" tests with cod liver oil and "negative control" tests with peanut oil gave the respective anticipated responses.

The eye conditions obtaining in some of the animals are shown in figure A: *1* shows rat C4033 after having been fed 1 gram of crop-milk daily for 18 days; *2* shows rat C4032 and illustrates a complete cure of a precious severe ophthalmia by the administration of 4 grams of crop-milk daily for 8 days;

3 and *4* show rat C4060 before and after the administration of pigeon egg yolk, and of whole pigeon egg, for 6 days, and illustrates the marked improvement that resulted.

In general these experiments may be taken to indicate the presence of vitamin A in crop-milk; 4 grams of this material are approximately as effective as 1 drop (30 mgm.) of cod liver oil for the cure of xerophthalmia and the promotion of growth in rats on an otherwise adequate diet. Pigeon egg yolk contains vitamin A in appreciable quantities.

Vitamin B (B + G) tests of crop-milk. Twelve male albino rats, weighing approximately 40 grams at weaning, were given a basal diet consisting of casein 35 per cent, starch 41 per cent, lard 20 per cent, and Osborne-Mendel salt mixture 4 per cent, supplemented by 2 drops of cod liver oil daily to supply vitamins A and D. When the animals failed to gain in body weight the ration was further supplemented as shown in table 3. The results of the feeding tests are taken to indicate that 3 grams of crop-milk supplied approximately the amount of vitamin B (B + G) contained in 0.1 gram of good brewery yeast.

DISCUSSION AND SUMMARY

The nutritive properties of the crop-milk produced by pigeons maintained on a standard mixed grain ration have been investigated; the content of protein, fat, ash, and water was 18.8 per cent, 12.7 per cent, 1.6 per cent, and 64.3 per cent respectively; the material therefore supplies approximately 190 calories per 100 grams. Feeding tests on albino rats have demonstrated the presence of vitamins A and B (B+G) in relatively low concentration; 4 grams of crop-milk were about equal in anti-ophthalmic power to 1 drop of a good grade of cod liver oil, and 3 grams of crop-milk promoted growth at approximately the same rate as 0.1 gram of dried brewery yeast. When fed as a supplement to rats on an otherwise adequate ration crop-milk is therefore a meager source of vitamins A and B (B+G); nevertheless the total quantity of these vitamins presented to the squab, which ingests crop-milk as its sole food, is relatively great. For example, a squab weighing 10 to 13 grams at hatching may ingest 3 to 4 grams of crop-milk the first day, 5 to 10 grams the next, and 10 and 12 grams the following 2 days. The squab has then received the equivalent of 1 drop of cod liver oil and 0.1 gram of dried yeast on the first day, 1.5 to 2 drops of cod liver oil and 0.2 gram of dried yeast on the second day, and 2.5 drops of cod liver oil and 0.4 gram dried yeast on the third and fourth days—a generous allowance of vitamins for so small a bird. Another source of vitamin A for the newly hatched pigeon is the pigeon egg yolk (demonstrated to contain appreciable quantities of vitamin A) which is folded into the gut at hatching.

The liberal supplies of vitamins received by the squab through the daily

ingestion of crop-milk are believed to contribute to the very rapid growth exhibited by these birds.

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ON THE EMPTYING OF THE GALL BLADDER

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While it seems clearly established through the work of Boyden (1925) and others that some evacuation of the gall bladder occurs after a suitable meal, there is a difference of opinion concerning the mechanism responsible for this. On the one hand it is assumed that the important factor is a lowering of pressure with which the full gall bladder has to contend, a pressure regulated through valves in the biliary ducts; or an increase in extra gall bladder pressure due to volume changes in adjacent organs. On the other hand it is believed that the gall bladder through its smooth muscle wall actively contracts and so empties itself. The evidence for the latter belief is based on results from a number of experimental procedures. Thus, it has been shown that isolated surviving gall bladder strips suspended in Ringer's or Locke's solution contract or relax when various drugs or other substances are added to the solution. Again the pressure in an isolated or intact gall bladder may be made to rise appreciably following the administration of smooth muscle stimulants. Finally by direct visual examination, by photographs or by x-rays, it is claimed that actual contractions may be demonstrated.

EXPERIMENTAL METHODS AND RESULTS. In the work reported at this time it was determined what degree of evacuation followed a fat rich meal in cats and whether this could be modified by drugs or other substances assumed to influence the gall bladder contractions. The meal was in most cases readily eaten. In the procedure adopted to measure the fullness of the gall bladder, the cat was killed by chloroform or a blow on the head, the cystic duct immediately clamped and the gall bladder removed. By means of a burette tied into the cystic duct, the amount of fluid added to the gall bladder contents to raise the pressure to a certain fixed level, was measured. A double burette as illustrated in figure 1 was found convenient for this purpose. The pressure used—viz., 100 mm. of water—was that determined by Mann (1919) as the minimum withstood by the common duct sphincter in cats. It is to be noted that there is very little difference in the readings we took at 75, 100, 120 and 140 mm. of pressure; and also that usually no further considerable distention takes place when fluid

is gradually added until 200 mm. of water pressure is reached. The total contents of the viscus were then measured. The amount run in from the burette gave the amount of emptying; and this compared to the total capacity of the gall bladder represented the emptying in per cent.

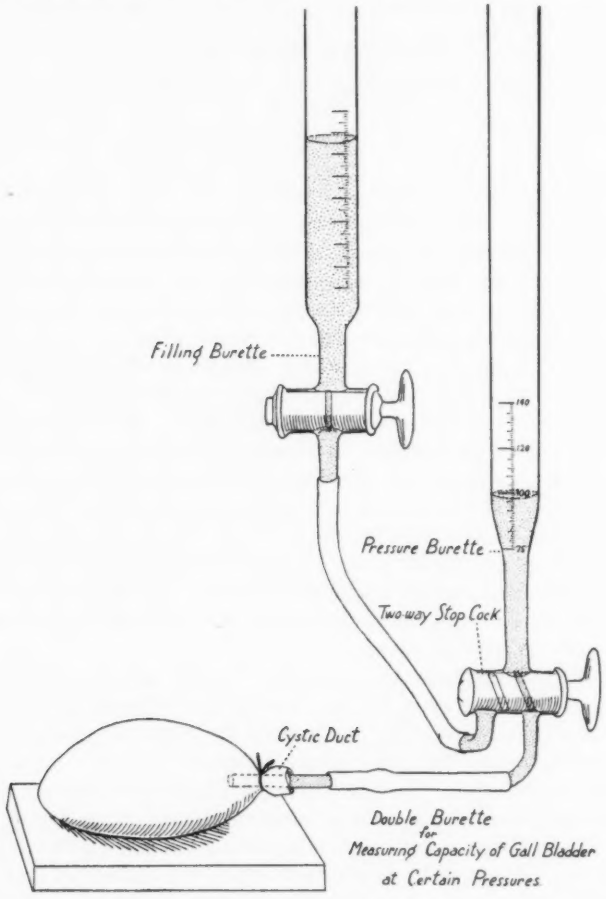

Fig. 1

It was found by this method that the gall bladders of cats not fed for 24 hours or more were filled to about $\frac{2}{3}$ of their capacity. At 4 hours more or less after a meal of 3 egg yolks and 60 cc. of cream, the gall bladders were about $\frac{1}{3}$ full, that is, about $\frac{2}{3}$ empty. When, accompanying the feed-

ing or during the 4 hour period previous to examination of the gall bladder, pilocarpine, atropine, epinephrine, ephedrine or papaverine, were given in single or repeated doses, insuring continued action throughout the period studied and in amounts which were pharmacologically effective, no appreciable change in the degree of emptying could be found either at the end of the 4 hour period or at any time previous. In unfed cats, pilocarpine or histamine in doses sufficient to produce salivation, vomiting, defecation and micturition, did not produce any emptying. The cholecystokinin of Ivy also did not cause any measurable emptying.

TABLE 1

Measurement of contents of gall bladders of cats

	GROUP 1 NOT FED FOR 24 HOURS OR MORE		GROUP 2 FAT RICH MEAL. GALL BLADDER MEASURED 4 HOURS AFTER MEAL	
	Number examined	Per cent empty	Number examined	Per cent empty
	3	0, 9, 14		
	11	27–38	2	31, 40
	2	43, 45	2	56
	3	60, 63, 63	19	66–90
Atropine			4	55, 68, 77, 83
Epinephrine			3	68, 71, 71
Ephedrine			1	31
			2	75, 81
Papaverine			2	40, 56
			3	88, 89, 89
Pilocarpine	2	36, 40	2	43, 57
Histamine	2	9, 19		
Cholecystokinin	3	2.5, 27, 30		

In the accompanying table the results are tabulated in two groups. The majority of the gall bladders in unfed cats, group I, are from 27 to 38 per cent empty. It is possible that in the last three of these the starvation period was not long enough. The majority of the gall bladders in the fat-fed cats, group II, are from 66 to 90 per cent empty. Only those cats were used in this series where fat absorption could be demonstrated by the appearance of the white chyle in the lacteals of the duodenum. These figures formed the basis for an analysis of this emptying, when drugs known to activate or inhibit smooth muscle (by acting on the muscle directly or upon its nerve supply) were given in doses shown to be effective in these experiments in eliciting the typical actions in each case. It is apparent that no emptying above the control level could be induced, nor could the emptying resulting from fat be either diminished or increased.

TABLE 2

Dose and manner of administration of drugs

DRUG	DOSE	MANNER GIVEN	TYPICAL ACTIONS ELICITED
		Cats	
Atropine	0.6 mgm.	Subcutaneous 30 minutes before meal and 90 minutes after	Response of cardiac and gastric vagi to electrical stimuli lost
Epinephrine (1–1000)	0.5 cc. + 0.25 cc.	Subcutaneous 0.5 cc. 10 minutes before meal; 0.25 cc. 45, 95, 170 and 200 minutes after	Gut relaxed Dose sufficient to produce glycosuria
Ephedrine	16 mgm.	30 minutes before meal	Gut relaxed (Kinnaman and Plant, 1931)
Papaverine	26 mgm.	Subcutaneous 10 minutes before meal in 2 cases	Gut relaxed
	25 mgm. +	25 mgm. subcutaneous 10 minutes before meal	No narcosis (Macht, 1917)
	12.5 mgm. + 12.5 mgm.	12.5 mgm. 60 minutes after and 12.5 mgm. 120 minutes after	
Pilocarpine	6–7.5 mgm.	Subcutaneous in controls	Salivation
		Subcutaneously 6–11 minutes after meal. Examined after 4 hours	Emesis Defecation Micturition Gut found tonically contracted with rings of constriction
Histamine	3 mgm.	Subcutaneous. Repeated in 30 minutes. Examined after 30 minutes	Salivation Emesis Defecation Micturition Gut contracted in rings
Cholecystokinin	12 mgm.	Intravenous Femoral vein prepared under local 3 hours previous. This is three times dose given by Ivy	

TABLE 2—*Concluded*

DRUG	DOSE	MANNER GIVEN	TYPICAL ACTIONS ELICITED
		Dogs	
Pilocarpine.....	3 mgm. 9–60 mgm.	Intravenously Subcutaneously	Marked tonic contraction rings in gut Salivation Emesis Defecation Pulmonary edema
Physostigmine....	1 mgm.	Subcutaneously	Vigorous peristalsis
Atropine.....	1 mgm.	Subcutaneously	Inhibited actions of pilocarpine and physostigmine
Histamine.....	0.25 mgm. + 0.75 mgm.	0.25 mgm. subcutaneously and 0.75 mgm. after 6 minutes	Marked peristalsis Tremors Defecation
Cholecystokinin..	6–60 mgm.	Intravenously	

Table 2 gives the dosage and the method of administration of the drugs and substances used. The doses in each case, though within physiological limits, were found to be adequate to produce typical effects. The dose of cholecystokinin was three times that used by Ivy.

It would appear, then, that these agents, which under certain conditions can cause contraction or relaxation of gall bladder strips or a rise in pressure in the exposed or the isolated gall bladder, fail to appreciably influence the emptying of the intact gall bladder of unanesthetized cats.

In another part of the work there was a direct visual examination of the gall bladder in unanesthetized dogs. The gall bladder, as well as any other abdominal organ desired, was brought into view by means of what may be called an abdominal endoscope. This instrument illustrated in figure 2 consists of a metal outer sheath of oval cross section accommodating in the upper $\frac{1}{3}$ of its long diameter a combined telescope and light carrier, while the channel formed by the lower $\frac{2}{3}$ allows of the passage of instruments such as electrodes, injecting needles, snares, biopsy punch, endothermy electrodes, etc.

Additional notations to supplement the drawing follow:

The outer sheath is 26 cm. long, oval in cross section with a caliber of 28 F. If no instrumentation is required the caliber of this sheath can be greatly reduced. The distal end is cut at an angle and blunted. The proximal end is fitted with a lock, 2 large stopcocks and a milled disk for holding.

The combined telescope and light carrier employs the McCarthy Foroblique lens system which gives, as the name implies, forward and oblique vision. The telescope is about the size of 14 F (F = French) and affords a brilliant field whose size varies with the distance of the object from the prism, placed just behind the 2.7 volt bulb.

With the endoscope sheath touching the object viewed, the field is 2.5 cm. in radius, so that by rotating the instrument on a perpendicular axis we can examine a field 5 cm. in diameter.

The current for the bulb is supplied by ordinary dry cells with an appropriate rheostat (a cystoscope or bronchoscope battery box does very well) through a flexible cord, which connects with the rotating contact clip.

The telescope is held in the upper third of the long diameter of the tube by passing through a rubber diaphragm in a hollow truncated metal cone, which fits into the lock. The lower portion of the diaphragm is perforated or not according to whether it is desired to introduce instruments through the channel formed by the lower two-thirds of the lumen. The diaphragm is held in place by a metal plate bearing pins

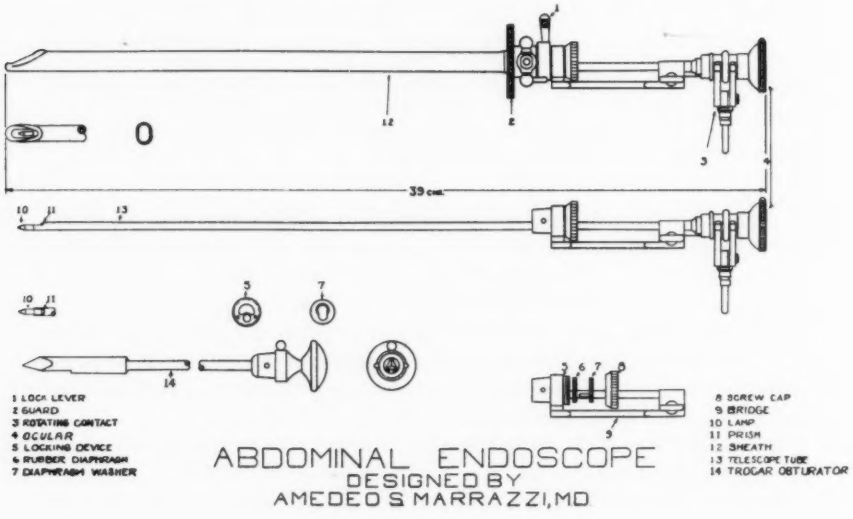

Fig. 2

fitting into the wall of the cone, and perforated with one or two apertures. The plate is held by a screw cap. The whole makes an air-tight joint.

The telescope projects beyond the sheath on its ocular side about 12 cm. to allow of the easy introduction of instruments while the eye is at the ocular. This projecting portion is held rigid by a metal bridge extending back from the cone and ending in an adjustable collar around the telescope just distal to the point of attachment of the rotating contact clip. A pin on the telescope also fits into the collar and prevents any rotation.

The obturator has a three-faced trocar tip with a slot cut out on one side to allow it to slip over the tip of the sheath into which it fits when in use.

Under local novocaine infiltration the endoscope is inserted through the abdominal wall into the peritoneal cavity.

The introduction of the endoscope comprises the following steps:

1. The abdomen of the animal, trained to lie quietly on its back or side

is prepared as for a sterile operation. The chosen site, preferably over some portion of the rectus abdominalis muscle, is infiltrated down to the peritoneum with 1 per cent novocaine. A $1\frac{1}{4}$ cm. skin incision is made. Next, a small amount of air, passed through a sterile cotton filter, is gently blown into the peritoneal cavity through a lumbar puncture needle pushed through the novocainized area. With this air cushion intervening between the anterior abdominal wall and the intestines, the sterilized outer sheath of the instrument, with the trocar-obturator filling the lumen, is carefully pushed through the subcutaneum, muscle and peritoneum, with a steady boring motion.

The telescope is now substituted for the trocar and a minimum of air is gently inflated through one of the stopcocks so as to lift the abdominal wall from the underlying gut and allow a clear view. When properly executed, this is all painless. With some training of the animal and with sterile precautions, observations can be made as frequently and over such periods as are desired, the animal lying quietly on its back and showing no evidence of discomfort. The gall bladder can be examined in any part and with the bright illumination stands out with great clearness.

In a description of the visible changes in the guinea pig's gall bladder following administration of food, Higgins and Mann (1926) report that this organ does not reduce as a unit, but that independent centers of contraction may be seen inducing a variety of patterns in the emptying vesicle. Gradually it assumes a hexagonal and then a pentagonal pattern. They saw no waves of contraction. In the dog, Mann (1932) has been unable to detect any visible contraction. On the other hand, Lueth, Ivy and Kloster (1929), observing directly the gall bladder in two dogs injected with cholecystokinin, saw a circular band of contraction appear at the midportion of the organ with some longitudinal shortening. Later, a similar but smaller band of contraction was seen which appeared and disappeared synchronously with tonus rhythm. It may be pointed out that these two reports describe quite different types of contractions and were made on animals with open abdomens.

In our experiments in which the endoscope was employed, the gall bladder was looked at with great care. Over thirty observations were made on a group of six trained dogs, and a thorough familiarity with the appearance of the organ was obtained. At varying times after a fat rich meal, there was evidence of some diminution in size of the gall bladder, in that there was a falling in of the fundus with some increase in the tortuosity of the vessels, but there were no signs of any actual contraction, either in form of contraction bands or in isolated areas, such as have been described.

Electrical stimulation, by electrodes passed through the tube and applied directly to the gall bladder wall, was without effect, as was also direct

pressure on the organ. Finally in a number of instances, the smooth muscle stimulants, pilocarpine, physostigmine and histamine, were administered in doses sufficient to produce very striking intestinal contraction without gall bladder effect. Cholecystokinin, kindly supplied by Doctor Ivy, also produced no visible contractions.

Discussion. The lack of uniformity in studies on the emptying of the gall bladder appears to us to be largely dependent upon the differing methods employed; and has caused us, after a critical review of the various types of experimental procedures, to employ the two methods here reported.

The difficulty in accepting evidence obtained from isolated surviving preparations of the gall bladder lies not only in the obvious difference between such a gall bladder and one normally in situ, but also in the relatively enormous doses of drugs used. For example, 5 mgm. intravenously in a 15 kgm. dog gives a concentration of 1 to 250,000, while Halpert and Lewis (1930), who use smaller doses than most, have a concentration usually of from 1 to 8000 to 1 to 80,000. Moreover, the drug in such preparations is all available for the gall bladder, whereas only a fraction of that injected into the general circulation reaches it. Furthermore, all except Ischiyama (1925) and later Halpert and Lewis make use of strips rather than the whole viscus, thus failing to elucidate the problem of emptying, while the output recorded by the latter investigators is too small to be regarded by them as significant.

Experiments with the gall bladder in situ in intact animals, on the other hand, suffer greatly from the necessity of subjecting the animal to anesthesia and to the operative trauma of exposure and of cannulating or inserting a balloon into the gall bladder or ducts with concomitant injury to the cystic artery and the nerves running along the ducts. At the same time, the intra-abdominal pressure relations are changed from normal by opening the abdomen.

The results are mainly in terms of pressure variations, which can be recorded in curves showing quite striking rises or falls of short duration. But they actually represent an output of not more than 1.2 cc. and usually about 0.75 cc., according to corresponding experiments of ours. They are further complicated by the action of these drugs upon the liver, and the influence of changes in liver volume upon the gall bladder has been clearly demonstrated by Bainbridge and Dale (1905).

In terms of emptying, then, this type of experiment, excepting in the study of response to fat meals, yields little information and no analysis of the mechanism. It nevertheless appears to be true from the work of Bainbridge and Dale (1905), Taylor and Wilson (1925), Burget (1927) and ourselves (unpublished) that changes of gall bladder tone may be produced by drugs in experiments where the influence of the liver is excluded by

stripping the gall bladder from its fossa down to the cystic duct. The changes, small in extent and transitory, do not so far appear to account for the emptying.

The drawback of reaching conclusions by the methods thus far discussed is illustrated, for example, when contractions of the exposed gall bladder in the intact dog recorded in Ivy's laboratory could not be confirmed in the same laboratory in the isolated surviving organ of the same species, although success is reported by his co-workers, Jung and Greengard (1932), with the isolated guinea-pig gall bladder.

Visualization has, of course, always been desired, and the intravenous dye and x-ray method has been developed to a considerable degree of clinical satisfaction. From the standpoint of physiological investigation, however, it leaves something to be desired. The pictures are at best difficult to interpret. They are, moreover, taken in one plane only, so that changes in other planes, though not otherwise noted, may modify the contour of the shadow appearing on the plate without necessarily denoting a change in volume, thus allowing of misinterpretation.

Lipiodal injection directly into the gall bladder has obviously the disadvantages attendant upon an operative procedure, including trauma of the gall bladder wall. Further, the usual lipiodal is of a higher specific gravity than gall bladder bile, thus complicating the picture by introducing the element of gravity as well as of a higher viscosity.

The definition afforded by fluoroscopy is so vague that, coupled with the objections to x-ray procedure stated above, we can attach little weight to the few reported positive observations: an equivocal one by Pendergrass, Overhalt and Ravdin (1931), who speak of having observed "what we believed to be rhythmic tonic contractions in a human gall bladder" (one case), but then state "It can be said that in these experiments we did not actually see the gall bladder contract," and in the dog, one by Potter and Mann (1926) and one by Ivy and Oldberg (1928).

It was natural despite all difficulties to attempt direct observations of the exposed viscus. This was undertaken by Higgins and Mann (1926) in locally anesthetized animals as already related. They were unable, however, according to Mann (1932), to detect any visible activity of the dog's gall bladder, studied in this fashion.

Ivy and his associates are, therefore, alone in their two reported observations of contractions of the exposed gall bladder in the dog (anesthesized). These are quite different from each other. In one case, when the cystic duct was clamped and the fundus cannulated, contraction bands are described. In another dog whose gall bladder was untouched, much the more physiological experiment, no bands were seen but a gradual reduction in size is reported.

It must be remembered that the gall bladder undergoes a wide excursion

with respiration, with an accompanying distortion of outline resulting from accentuation of the bend normally present at the junction of the neck with the cystic duct, as well as from deformities imposed upon the bladder by taut areas in its peritoneal covering, the occurrence and position of which are very irregular. Again, when the gall bladder is not completely full and is also lax, the contents move within it during its respiratory excursions, causing an undulatory rise and fall of the wall much like a wave of contraction. We mention these deceptive changes, which are artefacts, because others have informed us that their first impression (to be later corrected) at witnessing these changes was one of having seen definite contractions. Another factor to be borne in mind in evaluating such visual observation is the presence of a marked pulsation transmitted from the underlying vessels. Ivy's published photographs and observations are not convincing because of these facts, and because they are made after manually retracting the liver and adjacent viscera. The slightest change in the tension exerted by the retracting hand on the liver, for example, is readily reflected in the gall bladder which is embedded in and attached to it, and would upset any estimation of size or shape as well as comparison with a control photograph.

Our observations, though they differ markedly from those of Ivy and his associates, confirm the work of Friedenwald, Martindale and Kearney (1922) who were unable to observe any active contraction of the gall bladder in 34 dogs under various stages of anesthesia.

The necessity of working with preparations that are less close to the physiological than desired may be largely obviated by the methods of visualization in unanesthetized dogs and the quantitative estimation of gall bladder emptying in previously undisturbed animals that we have reported.

We wish to emphasize that the drugs used in these experiments in attempts to modify the emptying of the gall bladder were given in doses which proved adequate to produce their typical effects to a striking degree. Cholecystokinin was given up to 10 times the effective dose as assayed by Doctor Ivy. The claims made for cholecystokinin have not as yet been confirmed in published reports outside of Ivy's laboratory.

Though muscular activity has been very convincingly shown to play an important rôle in some animals (e.g., the fish), this is not necessarily to be expected in dogs. Thus an examination of the different species used in gall bladder investigation reveals a striking progressive diminution in visible activity from the lower to the higher animals or mammals. Higgins (1928) has reported definite peristalsis of the fish gall bladder; in the guinea pig, Higgins and Mann have seen the distinctly different picture of independent centers of contraction, gradually inducing a hexagonal and then a pentagonal shape in the emptying viscus; while in the dog, they

have been unable to demonstrate contraction visually. In the case of the monkey, Halpert (1927) points out that the gall bladder is normally almost completely embedded in the liver and could not undergo any very striking contractions.

That the dog's gall gladder is not devoid of the possibility of muscular change is indicated by the spontaneous rhythm recorded after cannulization of the gall bladder—freed from the liver—by Bainbridge and Dale (1905), Taylor and Wilson (1925), Burget (1927) and repeated by ourselves, as well as by the slight tonic response elicited by excessive doses of drugs such as pilocarpine. Such type of muscular activity, in its lack of perceptible motion, in its failure to respond to mechanical stimuli (pressure and needle puncture), to electrical stimuli and to otherwise effective doses of drugs influencing smooth muscle, seems very different from that smooth muscle activity, whose function elsewhere in the body is to produce emptying of a hollow viscus. It would seem, therefore, to play a very minor rôle normally in emptying the gall bladder. Its activity appears rather to partake of the nature of a variation of tone which may serve, as suggested by Graham (1926) and Halpert (1929), to effect adjustment of the gall bladder to its varying content. The last is borne out by the appearance of the gall bladder as we have followed it through its cycle of full, tense and smooth; partly empty, lax and wrinkled; same degree of emptying, but not wrinkled and not lax.

SUMMARY AND CONCLUSION

1. The extent of emptying of the gall bladder has been studied by a method of quantitatively estimating the gall bladder bile evacuated in normal, unoperated, fat-fed cats.

2. Gall bladder emptying was studied in unanesthetized trained dogs by a new method of visualization (abdominal endoscopy).

3. The mechanism of emptying has been further studied by means of drugs and substances known to affect smooth muscle directly or through its nerve supply, and by mechanical and electrical stimulation.

4. Direct observation of the viscus during a period when it is emptying failed to show any muscular contraction. An emptying of the gall bladder was not produced or influenced by drugs which have a definite effect on smooth muscle activity, nor by mechanical or electrical stimuli. The conclusion seems warranted, therefore, that muscle contraction in any way comparable to that occurring in other hollow organs, plays little or no part in this emptying.

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THE EFFECT OF CARBON DIOXIDE, HYPERVENTILATION, AND ANOXEMIA ON THE KNEE JERK

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In studies on the lower spinal reflexes reported in an earlier paper (King, Blair and Garrey, 1931), we encountered some evidence that the centers for these reflexes are affected qualitatively by an increase of the carbon dioxide tension in the blood and by anoxemia, as is the respiratory center. We are reporting in this paper the results of studies carried out for the purpose of more fully investigating this point, and also of throwing more light on the mechanism involved in the effects of acid-base changes in the blood on the lower spinal centers, and of evaluating the importance of these changes in accounting for spinal reflex variability within physiological limits.

We have adopted the knee jerk as the criterion, and have studied quantitatively the effects of increasing and decreasing the tensions of carbon dioxide and oxygen in the blood on this reaction. The experiments were carried out to a large extent on barbitalised dogs, but a few decerebrated animals were used. In one group the spinal cord was left intact, but in the other it was sectioned at the level of the last thoracic or first lumbar segment. In about half of the spinal animals the tests were made as soon as a brisk knee jerk could be obtained after the section, but in the others the transection of the cord was done under aseptic technique, and the animals used from four to twelve days later.

Inasmuch as no new principles were involved in the methods of elicitation of the knee jerk and its recording, it is needless to enter into a detailed description of the procedures. Suffice it to say that adequate steps were taken to secure immobilization of the thigh, and also for the control of the stimulus, both as to rate and intensity. The intensity of the stimulus employed was intermediate between minimal and maximal. The few exceptions will be indicated in the text. The blood samples were drawn through a cannula, either from the carotid or from the femoral artery. The respiratory gases were analysed by means of a modified Haldane gas analyser, the blood gases determined by the Van Slyke-Neill manometric method (1924), and the pH by the Cullen (1922) colorimetric method.

THE EFFECTS OF THE ADMINISTRATION OF CARBON DIOXIDE ON THE KNEE JERK. Before quantitative experiments were carried out, it was frequently observed on animals with the spinal cord intact, that the inhalation of a small volume of carbon dioxide resulted in a marked diminution or sometimes in a complete abolition of the knee jerk, while in the spinal animals the result of such a procedure was either no change in the knee jerk (fig. 1), or often an augmentation, and that in order to bring about a complete abolition of the reflex the administration of the gas had to be greatly prolonged. We then inaugurated a series of experiments which we hoped would enable us to arrive at a quantitative expression of this difference.

The carbon dioxide tension of the alveolar air and of the blood was raised by having the animal breathe known percentages of the gas from a Tissot spirometer, and also by rebreathing into a rubber bag of about three

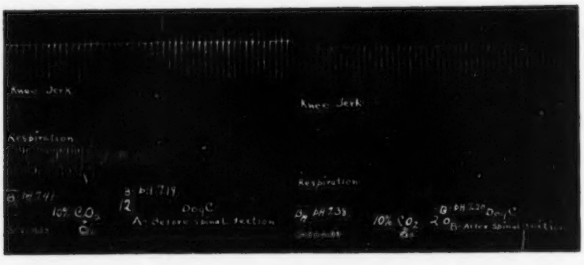

Fig. 1. The effect on the knee jerk of breathing 10 per cent carbon dioxide in oxygen. A, before spinal section, and B, after spinal section. The two records were taken from the same animal (dog C).

liters capacity. On the whole, the experiments in which the animal breathed from a spirometer and exhaled into the room were the most satisfactory. Under these conditions an equilibrium between the blood and alveolar gases is soon established and is maintained at a fairly constant level, while in the case of rebreathing, the level is constantly changing. The animals were allowed to breathe a definite volume of gas, in most of the experiments twelve liters, or the volume necessary to bring about the desired effect on the knee jerk was measured. Blood samples were taken synchronously with the various stages of the reactions obtained. We are fully aware that at any given instant the blood picture with reference to acid-base conditions may not accurately reflect the conditions in the tissues, but it is the most accessible and the best criterion we have, and if the samples are taken in succession and the procedure frequently repeated, the composite picture will at least indicate the trend in the tissues.

The experiments were begun with 2 per cent carbon dioxide in air. In

none of the animals breathing twelve liters of this gas was there any definite change in the knee jerk, although there was a slight increase in ventilation. Three per cent carbon dioxide in air was then tried. In the experiments of Bryan and Garrey (1931) a ten minute period of breathing 3 per cent carbon dioxide in air by a dog in parathyroid tetany resulted in a marked diminution of the clonic movements, but did not abolish the fibrillary twitchings. In our series the effects of breathing twelve liters of 3 per cent carbon dioxide in air were too slight and inconstant to permit any definite conclusions, but there was in every instance a definite increase in the rate and amplitude of respiration. All the gas mixtures containing more than 3 per cent carbon dioxide were enriched with oxygen, in order to guard against an anoxemia entering in as a complicating factor. With the breathing of such a mixture containing 4.5 per cent carbon dioxide in a number of animals with the spinal cord intact there was definite decrease in the knee jerk, but in none of the spinal animals was there any demonstrable effect on this reflex. With further increases in the carbon dioxide tension in the inspired air, in animals with the spinal cord intact, the diminution in response to the patellar tap became progressively more pronounced, so that with a gas containing 8 per cent of carbon dioxide the knee jerk was completely abolished in six out of eleven tests, and with 12.8 per cent it was completely abolished in every instance. In two of these animals a slight but definite augmentation of the knee jerk was recorded just preceding the diminution.

In the experiments with the spinal animals the effects of 2 and 3 per cent carbon dioxide were identical with those obtained on animals with the cord intact, no definite change occurring in the knee jerk, but there was some increase in ventilation. With the breathing of 4.5 per cent carbon dioxide in oxygen, the difference in reactions between the two groups of animals began to be in evidence. In the group with the cord intact, the breathing of 4.5 per cent carbon dioxide resulted, in a significant number of cases, in a depression of the knee jerk, but after transection of the cord there was no change in the reflex. In order to bring about a complete abolition of the knee jerk in the spinal animals, much higher percentages of carbon dioxide were required than in those with the cord intact, the percentages ranging from 9.8, the lowest, to 42, the highest. The type of reaction was also different in more than half of the experiments. Instead of a purely depressant effect on the knee jerk as was the case, with two exceptions, in animals with the cord intact, in more than half of the experiments on the spinal animals a stage of augmentation of the reflex appeared. In some cases there was only augmentation, variable in degree, in others the increased response was followed by a gradual depression. When no augmentation occurred the effect, if any, was one of depression, coming on gradually, and lacking the abruptness characteristic

of the reaction in the series with the cord intact. Figure 2 is illustrative of the augmentatory effect without depression. Another marked difference was noted between the animals of the two series. In those with the cord intact, during the initial stages of depression, and in some at the complete disappearance of the knee jerk, the usual procedures for reenforcement, such as the stimulation of a contralateral nerve, were

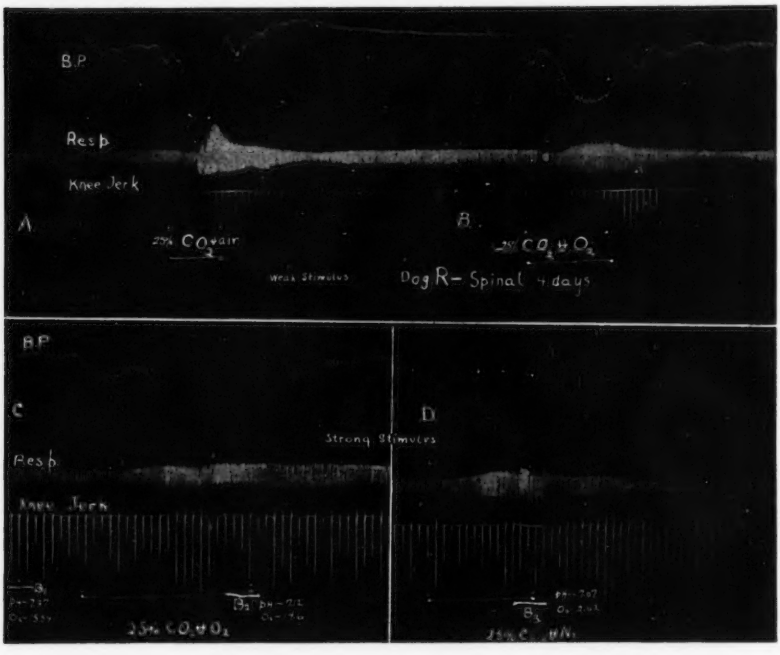

Fig. 2. The augmentatory effect of carbon dioxide on the knee jerk, shown in records A and B, using a stimulus just above the threshold. In records C and D a stimulus near the maximal was employed.

effective, but in the spinal animals they were less effective or failed entirely.

The results of the blood studies revealed very little which could not have been predicted from the analyses of the respiratory gases. They did, however, enable us to separate and discard those experiments in which the carbon dioxide changes were complicated by marked anoxemia, and furthermore threw some light on the cause of the variability between different animals, and also the variability in the same animal as the experiment progressed. No attempt was made to secure uniformity in the

condition of the animals by a course of preliminary care and feeding, consequently the initial control blood samples showed a wide variation in oxygen capacity, pH, and in base. On the basis of our data, no definite pH or carbon dioxide tension level can be correlated with the augmentation, depression, or complete abolition of the knee jerk. The changes indicated in figures 1 and 2 are representative of the average results, and show the differences between the spinal animals and those with the cord intact. Referring to figure 1, dog C, it is seen that before spinal section a change in pH from 7.41 to 7.19 was associated with an almost complete disappearance of the knee jerk, and that after spinal section a drop in pH of almost the same magnitude, from 7.38 to 7.20, was without effect on the reflex. In the records from dog R (fig. 2), the cord having been sectioned four days previously, a change from a pH of 7.37 to 7.12 (section C) shows no alteration in the knee jerk, while the same procedure (section B), with a stimulus just slightly above the threshold, shows a definite augmentation. On the whole, in animals with the cord intact, a drop of from 0.15 pH to 0.20 pH was necessary to bring about complete abolition of the knee jerk, while in the spinal animals a change approximately twice as great was necessary.

The results of these experiments indicate that the initial depression of the knee jerk in animals with the spinal cord intact, on raising the carbon dioxide tension of the alveolar air and of the blood, is not primarily due to a direct depression of the centers in the cord, but is dependent upon an effect on centers situated higher up in the neuraxis. They also indicate that the initial effect of raising the carbon dioxide tension in the blood is to increase the excitability of the lower spinal centers, but not to a degree comparable with the effect on the respiratory center. The depressant effect on the knee jerk, when the carbon dioxide tension is raised to a very high level, a result common to both groups of animals is, in all likelihood, due to direct depression of the lower spinal centers.

The assumption that the initial depression of the knee jerk in animals with the spinal cord intact, resulting from the administration of carbon dioxide, is not a direct one, raises the question as to the mechanism involved. With the data at hand the answer cannot be final. Presumably the effect is due to inhibitory influences from centers high up in the neuraxis, which may be excited either directly or reflexly. In order to throw light on these possibilities, studies were made on decerebrated animals, and on animals after double vagotomy.

In carrying out the decerebration the skull was trephined, then with a knife the brain was transected. On autopsy it was found that the line of division was fairly uniform, passing through the brain stem between the superior and inferior colliculi. The four animals thus prepared manifested considerable rigidity, or were easily thrown into the rigid state. On ad-

ministering air rich in carbon dioxide to these animals, the knee jerk behaved as it did in the spinal animals, not showing any sharp early diminution, but an augmentation in some cases, and disappearing only with the higher concentrations or after prolonged administration. These results indicate that carbon dioxide has a primary excitatory effect on centers, perhaps cortical, which in turn exert an inhibitory influence on the lower spinal centers. On decerebration the lower centers are released from these effects.

The possibility of another inhibitory factor must be taken into consideration, although it may play only a minor rôle. We have shown in earlier work (1931), and it also has been reported by others (Johnson and Luckhardt, 1927), that stimulation of the central stumps of the vagi in dogs exerts a marked inhibitory effect on the knee jerk. We have also demonstrated that under certain conditions there is a definite augmentation of the knee jerk synchronous with inspiration. The response to an increase in the carbon dioxide tension in the blood on the part of the respiratory mechanism is an hyperpnea. Thus, following the administration of carbon dioxide, two factors are released as the result of the increase in respiratory activity, one, an irradiation of impulses from the respiratory center which has an augmentatory effect on the knee jerk, and the other, an increase in afferent impulses from the lungs by way of the vagi, which have an inhibitory effect. It is not unreasonable to assume that under certain conditions the inhibitory effects brought about through the vagal afferents may balance or even outweigh the augmentatory effects from the respiratory center itself. This hypothesis receives support from our observations that the initial depressant effect of raising the carbon dioxide tension in the blood has, on the whole, been more marked in animals with the vagi intact.

The possibility of relations between variations in the knee jerk and circulatory changes has not been overlooked. Records of carotid blood pressure were taken in the majority of our experiments. The mean carotid blood pressure in our animals ranged from about 100 mm. to 140 mm. of mercury, and it was only when the pressure fell to very low levels, 30 mm. to 50 mm., and was maintained at that level for some minutes, or kept gradually falling, that any definite change in the knee jerk appeared. Under these conditions the reflex became increasingly more difficult to elicit. Our results indicate clearly that the variations in the knee jerk which we have described, resulting from the administration of carbon dioxide, cannot be correlated with variations in the mean arterial blood pressure. This is in keeping with the findings of Porter (1912) who studied the variations in the irritability of the spinal centers under asphyxial conditions.

THE EFFECTS OF HYPERVENTILATION ON THE KNEE JERK. Along with

the studies on the effect upon the knee jerk of an increase of the carbon dioxide tension in the blood, we have also studied the effects of its decrease. In order to carry this out we resorted to the expedient of hyperventilation. On the basis of a rather extensive literature it is evident that associated with an alkalosis, some parts, at least, of the central nervous system manifest a considerable degree of hyperactivity. Grant and Goldman (1920) were able to induce severe tetany in some human subjects by hyperventilation, Rosett (1924) reported similar findings, and Lennox and Cobb (1928) state that in epileptics a seizure can often be precipitated by the induction of an alkalosis. In our own laboratory, Bryan and Garrey (1931) were able to bring on violent tetany in parathyroidectomised dogs by this procedure. Our main interest has been to determine the effects of hyperventilation upon the knee jerk, using a stimulus of constant intensity.

In these experiments, just as in those described in the preceding paragraphs, two groups of animals were used, in one the spinal cord was left intact, and in the other it was sectioned. In order to carry out the hyperventilation, the animals, after barbitalisation, were placed into an airtight chamber enclosing the head, thorax, and the upper half of the abdomen. The tracheal cannula was connected to a short tube sealed into the wall and leading to the outside of the box. The hind quarters were drawn through an opening in a heavy rubber diaphragm, thus making these parts easily accessible for the study of the knee jerk. The interior of the chamber was then connected with a suction pump and a pressure tank through a system of dual valves operated by a motor driven speed reducer, timed, so that a fixed degree of negative pressure could be alternated with an equal degree of positive pressure. By the proper timing and the adjustment of the fluctuations of pressure within the chamber, overventilation could be carried out without a fall in blood pressure of sufficient magnitude to account for changes in the knee jerk.

The results of our experiments on animals with the cord intact were quite variable; in some instances the hyperventilation resulted in no change in the knee jerk, at other times a definite augmentation was obtained, and then again, a definite diminution, at times coming on very sharply with the beginning of the hyperventilation, and sometimes developing gradually (fig. 3). Numerous blood samples were drawn at various stages of the experiments, and determinations of the pH, carbon dioxide content and tension, and oxygen saturation made. The blood pictures of the acid-base conditions and the oxygen saturation were quite uniform, and showed no correlation with the knee jerk. The results with the spinal dogs were more uniform, and while there was no quantitative parallelism between the degree of alkalosis and the knee jerk, yet in the majority of instances the reflex was augmented under these conditions.

A number of parallel observations were made which may contribute to

the explanation of the variability in the knee jerk during and immediately following hyperventilation. In the first place, in a number of instances, an abrupt diminution in the knee jerk set in with the beginning of the artificial ventilation, and then just as abruptly returned to the original or higher level when the ventilation was stopped. In the second place, while we were able to produce tremors and tetany in less than half of our animals, in no instance did this condition appear posterior to the level of the spinal

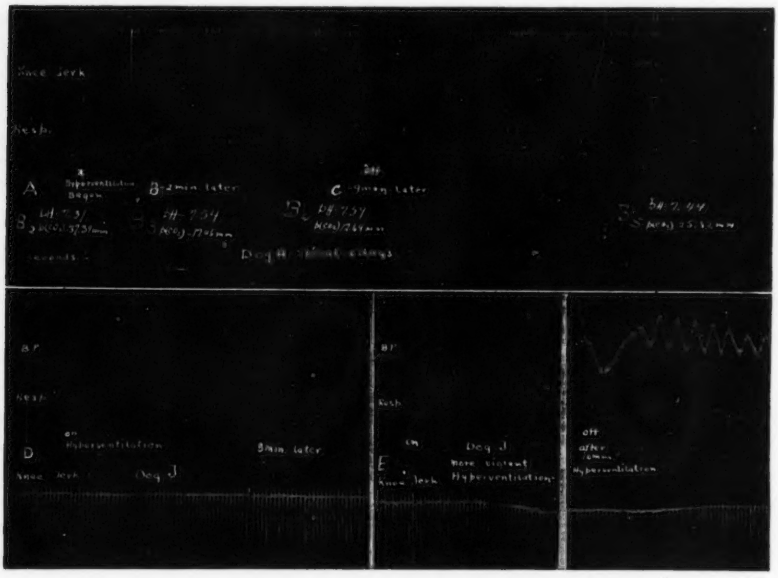

Fig. 3. The effect of hyperventilation on the knee jerk. Sections A, B, and C, taken from a spinal animal, show an augmentation. In D, from an animal with the cord intact, and with mild hyperventilation, no definite effect is shown. In E, taken from the same animal as D, but with more violent ventilation, a diminution is shown. In this case the extensor tone was increased, as shown by the drop in the base line. At B2, B3, B4 and B5 blood samples were drawn.

section. In the third place, the two decerebrate dogs (without spinal section) studied were easily thrown into tetany and rigidity by hyperventilation, and uniformly responded with an augmented knee jerk. These experiments, however, were usually of short duration, because with the onset of rigidity and the maintenance of the rigid state, studies on the knee jerk were rendered practically impossible. In the fourth place, after sectioning both vagi the effects of hyperventilation on the knee jerk were more uniformly in the direction of augmentation, but not always so.

These results and observations indicate that the primary effect of hyperventilation on the spinal centers consists in an increased excitability, but of a mild degree. In this case, and throughout this paper, we have used the terms excitability and irritability in a general sense, in terms of response to a constant stimulus, rather than in terms of threshold, no attempts having been made to determine the least stimulus in any instance. In the intact animal, however, the situation is a complicated one. A state of increased extensor tone and rigidity may set in which will greatly limit the excursions of the leg and present the picture of a diminished response (fig. 3, section E), or the direct effects on the cord may be either reënforced or inhibited by impulses from centers higher up in the neuraxis, the end result being an algebraic summation of these effects, and hence will depend upon the dominant factor. We feel justified in the conclusion that hyperventilation directly leads to an augmented excitability of certain cortical centers, the impulses from which have an inhibitory effect upon the lower spinal centers. An effect of this kind would aid in accounting, in part at least, for the depression of the knee jerk in some animals, and for the fact that it is not possible to demonstrate tetany in all waking human subjects by hyperventilation, and also for the relative ease with which the decerebrate animal is thrown into rigidity and tetany.

Another group of factors, some augmentatory, and some inhibitory, and reflex in character, must be given consideration. Without having direct evidence, one assumes that the increased pulmonary excursions and changes in the great veins in the thorax during hyperventilation give rise to an increase in the number of afferent impulses by way of the vagi. Stimulation of the central stumps of the vagi results in a diminution of the knee jerk, consequently the diminution in the knee jerk sometimes encountered during hyperventilation may, in part at least, be the result of vagal afferent impulses. Whether the result is dependent upon the pulmonary vagal afferents alone, or also upon the cardiac and gastric paths is not known. Impulses from the stomach may have an augmentatory effect upon the knee jerk, for Carlson (1916) found the knee jerk augmented during hunger contractions. It is also possible that the skeletal musculature involved in the thoracic and abdominal movements incident to respiration, even if artificially stretched, may give rise to impulses which enter into the picture and play some rôle, most likely augmentatory in character.

Thus, in the intact animal, the final effect of hyperventilation on the knee jerk is the resultant of the interplay of a number of opposing factors. By decerebration, the cortical inhibitory factors are eliminated, while by spinal section both the cortical influences and those arising lower down are erased. With the spinal centers thus isolated, the effect of hyperventilation upon them is that of a mild augmentation of excitability.

While an augmentation of the knee jerk in the spinal animal cannot be

demonstrated in 100 per cent of the cases, on increasing the carbon dioxide tension in the blood and on hyperventilation, yet it occurs frequently enough to be of significance and, it seems to us, to warrant the conclusions we have drawn. We are thus confronted with a dilemma in which, either by increasing or by decreasing a given factor, the same end result is attained. Walshe (1923) has contended that the hyperexcitability of the central nervous system resulting from hyperventilation is due to tissue anoxia. If this were true, then the dilemma would vanish. His assumption is, that owing to the removal of carbon dioxide the oxygen is not released from the hemoglobin, consequently the tissues actually suffer from oxygen deficiency, and become more acid. This would point to the H ion as the determining factor, being increased, on the one hand, by piling up of carbon dioxide, and on the other hand, as the result of the failure to oxidise lactic acid. Laying aside the improbability, on the basis of dissociation, of this hypothesis, we have accumulated evidence that hyperventilation is not associated with tissue anoxia. Many of our experiments were carried out with the animal breathing into a metabolimeter, and the oxygen consumption determined before, during, and after hyperventilation. If a tissue anoxia supervenes upon hyperventilation, it should be reflected on the oxygen consumption curve. In no instance have we recorded a decrease in oxygen consumption under these conditions, but on the contrary, in a few experiments, an actual increase. Tissue anoxia is therefore ruled out as an explanation, and in the absence of any other conclusive data, we must leave the question an open one.

THE EFFECT OF ANOXIA ON THE KNEE JERK. Sherrington (1910) observed that a slight degree of asphyxia facilitates the elicitation of the scratch reflex in the decapitate and in the spinal animal. Porter (1912) studied the effect of anoxia on the threshold for the flexion reflex on stimulation of the tibial nerve in the spinal animal, and found the effect predominantly depressant. Winkler (1929) reported an augmentatory effect on the reflex contraction of the anterior tibial muscle as the result of anoxia, but he used no spinal animals, and since he introduced a soda-lime cartridge into the respiratory circuit, it is quite likely that the anoxia was complicated by some degree of alkalosis.

We have made a study of the effects of various degrees of anoxia on the knee jerk of animals with the spinal cord intact and also with the cord transected; we have also controlled, within limits, the carbon dioxide tension in the blood during the period of oxygen lack. Instead of using a closed circuit containing a soda-lime cartridge, we prepared mixtures of nitrogen, oxygen, and carbon dioxide in a Tissot spirometer, from which the animal inhaled, and then exhaled into the room. By adding between 2 and 3 vols. per cent of carbon dioxide to the inspired gas, the blood changes in pH and in carbon dioxide tension were less than those found necessary to produce any demonstrable effect upon the knee jerk.

The effects of anoxia on the knee jerk in both groups of animals were predominantly depressant, but quite variable, and may be grouped under three heads,—initial, intermediate, and terminal. We were not able to demonstrate any definite effect on the knee jerk with gas mixtures containing 12 per cent or more of oxygen, but with 10 per cent there was some change in a few animals, and with 8 per cent and less there was always a definite effect.

In about 30 per cent of the experiments on animals with the spinal cord intact, and in about 45 per cent after sectioning the cord, the initial effect of anoxia was an augmentation of the knee jerk, mild and of short duration, but definite. These figures are slightly higher than those given by Porter (1912) who studied the effect of anoxia on the threshold for a flexion reflex, and found it lowered in eleven out of forty-seven cases. Since the duration of the period of augmentation is brief, and his tests were made at intervals

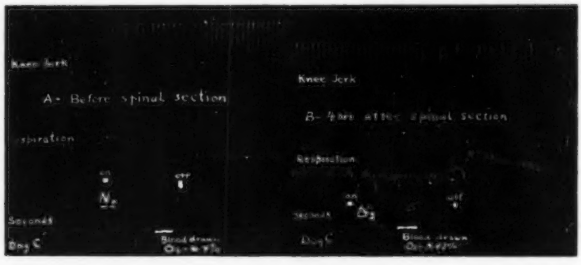

Fig. 4. The effect of moderate anoxia on the knee jerk. A, before spinal section, and B, after spinal section. Both records were taken from the same animal (dog C), and the anoxia was of about the same degree in both cases.

of several minutes, it is not unlikely that had he made continuous records or more frequent tests, he would have found a higher incidence of augmented excitability.

The intermediate stage was invariably one of depression of the knee jerk, ranging from a slight diminution in the reflex to its complete abolition. The degree of depression in any given experiment ran closely parallel with the severity of the anoxia. Blood samples were taken at various points, corresponding in time as closely as possible to various stages of the change in the knee jerk. The results of the analyses of these blood samples revealed the fact than an anoxia of greater severity is required to abolish the knee jerk in the spinal animal than in one with the cord intact (fig. 4), the average, on the basis of oxygen saturation, being 34 per cent for the intact animals, and 15 per cent for those with the cord sectioned. These results indicate that, just as in the case of raising the carbon dioxide tension

in the blood of animals with the spinal cord intact, impulses from centers situated higher up in the neuraxis enter into the picture, and play an important rôle in determining the effect of anoxia upon the knee jerk.

When the anoxia was pushed to the extreme, we frequently encountered the phenomenon which we have designated as the terminal reaction. At this stage the knee jerk was no longer elicitable with the stimulus previously effective, the blood pressure was rapidly rising, the respiratory efforts were slow and of the gasping type and soon ceased entirely. The arterial pressure kept rising for a few seconds after the cessation of respiration, then broke sharply and began to fall rapidly. When this stage was reached, a few of the animals, if disconnected from the Tissot and left to themselves, recovered, but in the majority the situation rapidly became more critical, and demanded the institution of measures for resuscitation, at times successful, and at other times, not. Almost synchronous with the beginning of the fall in blood pressure the knee jerk again became elicitable, rapidly increased in height for four or five responses, then declined in the same manner. At this time the animal usually made a few gasping efforts, and if it rallied from this point and recovered, the knee jerk soon again became elicitable and gradually recovered its normal intensity, but if the animal failed to rally, no more reflex responses were obtained. In so far as the knee jerk is concerned the phenomenon which we have just described is not dependent upon the integrity of the connections between the lower spinal centers and those higher up, for it occurred just as frequently in spinal animals as in those with the cord intact.

On the basis of our results, we feel that the augmentation of the knee jerk associated with the early stages of anoxia occurs frequently enough to be of some significance, and are justified in the conclusion that there is some increase in the excitability of the lower spinal centers, but not to a degree comparable with the effect of anoxia upon the respiratory center. If the anoxia is intense or prolonged, the final effect is always one of depression which may reach the state of complete reflex inexcitability. The recovery from this state is dependent upon the duration and the intensity of the anoxia. In the extreme cases which we have described, in which a brief paroxysm of knee jerks appears following the cessation of breathing and synchronous with the break in blood pressure, the interpretation and analysis are somewhat difficult. Blood samples taken at this stage have invariably shown a very low oxygen content, 0.92 vol. per cent being the lowest from which recovery took place, and a carbon dioxide tension higher than that found as the norm. We have also been much more successful in resuscitating these animals by the use of carbon dioxide and oxygen, than by the use of oxygen alone. Our hypothesis is, that the respiratory and spinal centers, as a result of the anoxia, become so depressed that they

no longer respond to the stimuli at hand. With the cessation of respiration the anoxia becomes more intense, but at the same time the carbon dioxide tension in the blood gradually mounts, and finally reaches a level which may be sufficient to set the respiratory center into activity and to raise the excitability of the spinal centers to the point where the patellar taps become effective stimuli. This is the critical point, and if the tissue acidity has not been too great the animal may gradually rally and recover. On the other hand, the tissues may have become so acid that the slightly added increase in the acidity of the blood due to the oxygenation of the hemoglobin, and of the tissues themselves as the result of their activity, renders recovery impossible.

SUMMARY AND CONCLUSIONS

1. In the dog with the spinal cord intact, the initial depression of the knee jerk which results from an increase of the carbon dioxide tension of the blood, is not due to a direct depression of the spinal reflex centers, but to an inhibitory effect from higher centers, due in part to their direct excitation by the carbon dioxide, and in part to reflex excitation.

2. The effect of hyperventilation on the knee jerk is that of a mild augmentation, but in animals with the cord intact, inhibitory influences from higher centers may dominate and lead to an actual diminution. The actual excursion of the leg may also be diminished because of the development of a state of rigidity and increased extensor tone. There is no evidence that the effects of hyperventilation are due to tissue anoxia.

3. In the early stages of anoxia there is evidence of a short period of increased excitability of the lower spinal centers. The effects of a severe and prolonged anoxia are always depressant.

4. The lower spinal centers are qualitatively affected by an increase in the carbon dioxide tension in the blood and by anoxia as is the respiratory center, but quantitatively are much less responsive.

5. The effects of acidosis, alkalosis, and anoxia, within physiological limits, play but a small rôle in accounting for the variability in spinal reflex responses, and are largely overshadowed by other inhibitory and augmentatory factors.

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THE NUTRITIVE VALUE AND EFFICIENCY OF MINERALIZED MILK[1]

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A discussion of the nutritive value of milk immediately brings to mind such common phrases as "milk, the perfect food" and "milk, the indispensable food." These statements are based on our knowledge concerning the general welfare of suckling mammals and upon certain observations made in the laboratory. The young of practically all mammals flourish reasonably well during the suckling period when milk is the sole source of food. When experimental animals are fed rations deficient in one of the basic constituents such as protein or one of the vitamins, milk in most cases has been found to supply this deficiency remarkably well. In fact the early experiments by Hopkins (1912) in which he obtained such extraordinarily good results when small amounts of milk were added to a diet of purified foodstuffs gave the real impetus to our modern studies on nutrition. Mann (1926) studying the importance of milk in the diet for boys between the ages of 6 and 11 years demonstrated that the group receiving one pint of milk in addition to a regular well balanced diet grew and developed much better than did the group not receiving the milk.

In spite of this exceedingly favorable attitude toward the nutritive value of milk, no one has been able, as far as we know, to rear a mammal from weaning to maturity on whole cow's milk alone. When rats are restricted after weaning to a diet of milk they grow for 3 to 4 weeks, reaching a weight of about 100 grams after which the animals decline in weight and die of anemia. The anemia which develops under these conditions has prevented the use of an exclusive milk diet for studying its nutritive properties. The inability of milk to produce hemoglobin was always attributed to its low iron content but Hart, Steenbock, Waddell and Elvehjem (1928) demonstrated that milk is also deficient in copper. This element acts as a supplement to iron in hemoglobin formation, and it must be added to milk as well as inorganic iron in order to prevent the development of anemia. When milk supplemented with iron and copper is fed to rats at

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weaning fair growth is obtained, or if it is given to rats already suffering from anemia, the rate of growth increases with the improvement in the hemoglobin of the blood. In either case the rate of growth is not optimum. Curves given by Krauss (1931) show that male rats fed milk supplemented with iron and copper from weaning required almost 15 weeks to grow from 60 to 200 grams. It is more difficult to follow the growth records of rats which were first made anemic and then cured with iron and copper because the animals were often discarded as soon as the hemoglobin regeneration was complete, but the curves given by Waddell, Steenbock, Elvehjem and Hart (1929) show that the rats rarely gained more than 100 grams in a period of 5 weeks.

More recently Kemmerer, Elvehjem and Hart (1931), working with mice, and Skinner, Peterson and Steenbock (1932), working with rats, have demonstrated that the addition of traces of manganese to a diet of whole cow's milk supplemented with iron and copper has a favorable effect upon growth. Since the iron, copper, and manganese can be added as inorganic salts, milk mineralized with these salts should give an excellent diet for studying the nutritive value and efficiency of milk. We are including in this paper the results obtained with rats and pigs when fed such a ration.

EXPERIMENTAL. The rats were reared according to the technique outlined by Elvehjem and Kemmerer (1931). When the young were 21 days old the male rats in each litter were placed in individual cages equipped with wire screen floors and they were fed only whole milk mineralized with iron, copper, and manganese. These elements were added as solutions of $FeCl_3$, $CuSO_4$, and $MnCl_2$ so that 30 cc. of milk contained 0.5 mgm. Fe, 0.05 mgm. Cu, and 0.04 mgm. Mn. Fresh whole cow's milk (Holstein) was supplemented with these solutions each morning and fed ad libitum to the rats immediately. A sufficient supply was placed in a refrigerator to give the rats an additional supply in the afternoon. The consumption was determined by measuring the amount of milk fed daily and the amount remaining at the end of each day.

In order to have comparable growth records for rats reared on an ordinary diet several litters were reared on shavings. When the young were 21 days old, the males were placed in individual cages on shavings and continued on the stock ration and whole milk. The rats were weighed once weekly. The weighings were made at the same time of day each week.

In table 1 are tabulated the number of days required to grow from 60 to 200 grams and the average daily gain for a few typical rats raised on each of the two rations. It is readily seen that the growth of the rats on the mineralized milk was exceedingly good and that they made equally as rapid gains as those fed on the stock ration. An average daily gain of almost 4.0 grams is exceptional compared to Donaldson's (1924) standards

which give an average daily gain of 1.8 grams for male rats during a similar period of life.

The average of 36 days required for the rats to grow from 60 to 200 grams is almost identical with the results obtained by Bryan and Gaiser (1932) for rats grown on Mendel's special diet composed of yellow corn, skim milk powder, casein, and salts, together with liver and lettuce. The average daily gain of 3.9 grams on mineralized milk does not compare with the rapid gain of 5.3 grams obtained by Mendel and Cannon (1927) and the phenomenal growth of 6.1 grams recently obtained by Anderson and Smith (1932). However, both of these results were obtained with the rats used at the Connecticut Agricultural Experiment Station. Undoubtedly the strain of rats used in that laboratory is much larger than those used in our

TABLE 1

Rate of growth of male rats on mineralized milk and on the stock ration

MINERALIZED MILK			STOCK RATION		
Rat no.	Time required to grow from 60–200 grams	Average daily gain	Rat no.	Time required to grow from 60–200 grams	Average daily gain
	days	*grams*		*days*	*grams*
133	35	4.0	400	38	3.7
136	36	3.9	401	38	3.7
173	36	3.9	402	31	4.5
174	38	3.7	403	36	3.9
203	37	3.8	404	40	3.5
334	33	4.2	405	34	4.1
Average....	36	3.9	Average...	36	3.9

laboratory because Anderson and Smith speak of males weighing over 700 grams while the males in our colony rarely reach 500 grams.

The efficiency of the mineralized milk was also found to be quite high. An average for a considerable number of rats shows that only 2.25 grams of milk solids were necessary to produce 1 gram of gain in weight. Although these results are of a preliminary nature, they do substantiate in a very definite way the conclusions which have been based previously on indirect evidence.

A similar experiment was conducted with pigs. Ten young Yorkshire pigs were selected from the general herd shortly after weaning and placed in individual pens in the University swine barns. The floors were of concrete and shavings were used for litter. We wish to emphasize, therefore, that the pigs were not kept under as restricted conditions as were the rats. The whole cow's milk fed was limited so that all pigs consumed daily the same amount. During the first 4 weeks each pig consumed 12

pounds of milk per day, during the second four weeks, 18 pounds, the third 22, the fourth 24, and during the fifth and sixth four week periods the consumption was 30 pounds per pig daily. A solution containing 50 mgm. Fe as $FeCl_3$, 5 mgm. Cu as $CuSO_4$, and 5 mgm. Mn as $MnCl_2$ was added to the milk of each pig every morning. Twenty-five cubic centimeters of cod liver oil were given once weekly to insure a sufficient supply of vitamin D. The pigs were weighed every 4 weeks.

TABLE 2

Growth of pigs on mineralized milk

WEEKS ON DIET	WEIGHT OF PIGS				
	No. 6	No. 7	No. 8	No. 9	No. 10
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
0	51	57	45	26	29
4	78	76	67	49	53
8	118	114	96	86	95
12	160	155	141	129	144
16	198	193	175	167	179
21	243	246	226	222	241
25	280	280	278	266	283

TABLE 3

Average gain of pigs on mineralized milk and a standard dry ration

WEEKS ON RATION	AVERAGE GAIN IN WEIGHT	
	Mineralized milk	Standard ration
	lbs.	*lbs.*
4	23.0	22.5
8	60.2	54.8
12	104.2	97.0
16	141.8	148.0

The pigs grew extremely well on this diet and appeared to be normal in every way. This diet apparently was very satisfying since the pigs appeared to be quiet and contented. The growth records of 5 typical animals are recorded in table 2. In table 3 the average gain in weight is compared with similar values obtained for a large group of pigs raised on a standard mixed ration consisting of yellow corn 77 parts, dried butter milk 14.5 parts, linseed meal 3 parts, alfalfa meal 5 parts, and iodized salt 0.5 part. Growth on the two rations is very similar. In fact during the first 12 weeks the pigs on the mineralized milk grew slightly faster than those on the dry ration. The pigs on mineralized milk have been raised

to maturity and reproduction is being studied at the present time. These results will be presented later.

The average daily gain of the pigs on mineralized milk during the first 16 weeks was 1.26 pounds. The gain is considerably greater than the average figure of 0.9 pound given by Henry and Morrison (*Feeds and Feeding*). The rate of gain would possibly have been greater had ad libitum feeding been practiced.

Upon calculating the efficiency of the milk ration and the dry ration, it was found that only 1.97 pounds of milk solids were necessary to produce 1 pound of gain, while 3.53 pounds of the standard ration were required for the same gain. These results demonstrate further the excellent nutritive value of milk alone when specific inorganic deficiencies in milk are corrected.

Discussion. The direct feeding of mineralized milk to experimental animals will undoubtedly inaugurate many new problems. We know that iron and copper do not need to be added to milk for very young animals because there is a sufficient supply of these elements in the liver to counteract the deficiency in milk. The supply, however, varies in different species and in different animals of the same species. It is important to determine when the inorganic supplements should be added in order to give the best results. The destructive action of these elements on certain organic factors in the milk is another question which must be given consideration. The rapid growth resulting from the feeding of mineralized milk increases the requirement of other nutritive factors in the milk. If these factors are present in low amounts they may limit growth and development.

If the mineralization of milk is to be used under practical conditions the simplest and most efficient method of supplying these elements must be worked out. These are problems for the future, and in this paper we are merely demonstrating the possible importance of mineralized milk in experimental work.

SUMMARY

Rats reared from weaning on whole cow's milk mineralized with iron, copper, and manganese grew from 60 to 200 grams in 36 days. The average daily gain of 3.9 grams was very similar to the gain made by rats on an ordinary ration. Two and twenty-five hundredths grams of milk solids were required to produce a gain of 1 gram in weight.

Pigs reared on mineralized milk (plus cod liver oil) made an average daily gain of 1.26 pounds over a period of 16 weeks. The rate of gain was practically identical with that made by pigs on a standard dry ration. Only 1.97 pounds of milk solids were necessary to produce 1 pound gain in weight while 3.53 pounds of the dry ration were needed to produce the same gain.

The nutritive value and efficiency of whole cow's milk has been demonstrated by direct feeding after the deficiencies in the milk were corrected by the addition of inorganic supplements.

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THE NATURE OF THE FOODSTUFFS OXIDIZED TO PROVIDE ENERGY IN MUSCULAR EXERCISE

IV. The Use of Protein as a Fuel in Exercise

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In previous papers of this series (Rapport and Ralli, 1928) evidence was brought forward to indicate that carbohydrate was not the exclusive source of oxidative energy in muscular contraction, but that muscles could utilize either carbohydrate, or fat, or both, depending upon the proportions in which these substances are presented to them in available form. No account was taken of the possibility that protein might also be used for this purpose.

Such opinion as exists on this point is based upon studies made upon the nitrogen metabolism, and particularly upon the presence or absence of a rise in the nitrogen excretion during and following muscular effort. It is well known (Pflüger, 1891) that the dog can exist on an almost purely protein diet, and there is inferential evidence that in the later stages of fasting, protein may contribute to the energy requirement of exercise.[1] Under ordinary conditions, however, evidence for the extensive utilization of protein in exercise is slight. On the question of whether muscular work is associated with a rise in nitrogen excretion opinion is divided. It is clear, however, that even with a large amount of work, the excreted nitrogen is at least not greatly increased, except perhaps after long continued starvation, and apart from this, nothing in this type of evidence indicates that a noteworthy percentage of the needed oxidative energy of muscular metabolism is derived from protein breakdown. This phase of the matter has been adequately reviewed by Cathcart (1925) and by Lusk (1928).

Nevertheless it seems to us that in a sense the study of the nitrogen excretion is somewhat irrelevant to the question of the utilization of protein energy. If one assumes that the nitrogenous fragment of deamination can not be used as a source of oxidative energy, then the matter

[1] In a paper reported by Dann at the meeting of the American Physiological Society, April, 1932, it was shown that the pre-mortal metabolism of a fasting dog was practically entirely protein, judging both from the nitrogen excretion and the respiratory exchange.

reduces itself to the utilization of the non-nitrogenous fragments, and studies of the nitrogen metabolism do not necessarily furnish evidence in regard to this utilization. For example, let us suppose that on an ample mixed diet with the subject at rest, the N excretion is 12 grams per 24 hours, indicating the apparent metabolism of 75 grams of protein. Let us further assume that with a heavy day's work the nitrogen excretion on the same diet remains at 12 grams. This proves no more than that. It may not even, as Cathcart has pointed out, prove that deaminization has been no greater, providing protein synthesis has simultaneously occurred. Waiving this point, however, it certainly throws no light upon the fate of the non-nitrogenous portion, in the sense of ruling out the use of the latter to provide energy for the muscular exercise on the day of work.

This question we have studied, using the excess respiratory quotient of exercise as a criterion of the non-nitrogenous metabolism. The results indicate that protein, as well as carbohydrate and fat, can be used as a fuel in muscular exercise.

EXPERIMENTAL PROCEDURE. The experiments were performed on a bitch weighing about eleven kilos, the respiratory metabolism being obtained by the use of a closed system of the Benedict-Homans type previously described in communications from this laboratory. Exercise was accomplished by causing the dog to run on a horizontal treadmill introduced into the animal chamber, as described in a previous paper (Rapport, 1929). The exercise was mild, consisting of 15 minutes' running at approximately 2.5 kilometres per hour. With this degree of exertion recovery, as evidenced by the R. Q. and the oxygen consumption, was usually complete within ½ hour. The experiment was ordinarily continued for 2 or 3 half-hour periods after this (longer if necessary), to establish the post-recovery resting level. The excess metabolism of exercise and recovery was calculated upon the difference between the gaseous exchange of exercise and recovery, and that of the average of the pre- and post-exercise resting periods. In a few instances this calculation was also made upon the pre-exercise period alone, when a possibility of difference in the interpretation of results made this desirable.

Our general method was to establish as nearly as possible an exclusive protein metabolism, and then to see whether during exercise the animal still continued to use protein as at rest. Our criterion of the amount of protein metabolism was the respiratory quotient, and since the quotient of a mixture of carbohydrate and fat can approximate that of protein, it was necessary for us first to create a one-sided metabolism of one or the other of these non-protein substances, in order to be able to differentiate this from the ensuing metabolism after giving protein. We attempted, therefore, to do the following: 1, to approach a theoretical respiratory quotient of 0.71 by fat feeding or starvation on days preceding the experi-

ment, or to approach a theoretical quotient of 1.00 similarly by carbohydrate feeding; 2, to give an amount of lean beef heart on the experimental day that would result in an exclusive or nearly exclusive protein metabolism at rest, as shown by a shift of the R. Q. to that of protein; 3, then to exercise the animal and see whether the R. Q. of exercise and recovery remained that of protein, or reverted to the prevailing quotient before the meat feeding.

The average nitrogen excretion per hour of the experimental period was determined and has been recorded. It must be emphasized, however, that this does not accurately represent the actual protein metabolism of the period, because of the delay in urea formation and excretion. As was shown in a previous paper (Ralli, Canzanelli and Rapport, 1931, 337), the nitrogen excretion may represent 50 per cent or less of the actual protein metabolism in an acute experiment after protein feeding, and this discrepancy is even more likely to occur in the short experiments reported in the present paper.

Four alcohol checks were performed during the course of the work, the individual respiratory quotients being 0.660, 0.656, 0,654, and 0.667, an average of 0.659.

EXPERIMENTAL RESULTS. *Protein feeding (a) after starvation and fat diet.* The results are summarized in table 1. After 48 hours of starvation (excepting 300 grams beef heart on the preceding day) the average resting R. Q. on October 8 was 0.74 and the excess R. Q. of exercise and recovery was the same. On the following day, after 24 hours more of starvation, 500 grams of lean beef heart were given, resulting in a resting R. Q. of 0.76 and an excess R. Q. in exercise and recovery of 0.79.

On October 10 and 11 a high fat diet, (including enough protein to maintain N equilibrium) was given, and on October 12 500 grams of beef were again administered. The resulting resting quotient before exercise was 0.79, which remained the same during exercise and recovery; but dropped to an average of 0.75 for the 4 half-hour periods following recovery. Calculated on the average resting metabolism before and after exercise, the excess quotient of exercise was 0.82. It would probably be more accurate to take the calculation based on the pre-exercise period, giving an excess R. Q. of 0.79, for it seems probable that in the later periods (4 to 5 hours after this meat feeding) the effect of the protein had begun to wear off. In either case, the evidence would indicate protein consumption during the exercise.

On the next day, October 13, the post-absorptive metabolism (no other food having been given) showed an R. Q. of 0.72, or practically that of fat. On giving 500 grams of beef, the resting R. Q. jumped to 0.79, indicating an almost exclusive protein metabolism, while that of exercise and recovery was 0.81, the excess R. Q. being 0.86.

TABLE 1

Excess R. Q. of exercise and recovery

DATE	MAINTENANCE RATION	EXPERIMENTAL DIET (BEFORE EXPERIMENT)	N EXCRETION PER HOUR	RESTING METABOLISM				EXERCISE AND RECOVERY	EXCESS R. Q.	
				Before exercise		After recovery	Average resting		Over pre-exercise resting	Over average resting
				Post absorptive	After food					
1931			*gram*							
Oct. 6	High carbohydrate* Oct. 5	None	0.078		0.93	0.89	0.91	0.91		0.90
Oct. 8	300 grams beef Oct. 7	None	0.110		0.74	0.74	0.74	0.74		0.74
Oct. 9	No food since Oct. 7	500 grams beef	0.339‡		0.77	0.76	0.76	0.78		0.79
Oct. 12	High fat† Oct. 10, 11	500 grams beef	0.405		0.79	0.75	0.77	0.79	0.79	0 82
Oct. 13	High fat † Oct. 10, 11; beef experiment Oct. 12	500 grams beef	0.367	0.72	0.79	0.77	0.78	0.81		0.86
Oct. 14	Beef experiment Oct. 13	None	0.209		0.76	0.73	0.75	0.77	0.78	0.83
Oct. 15	No food since Oct. 13	None	Lost		0.73	0.74	0.73	0.73		0.72
Oct. 16	High fat† Oct. 15	500 grams beef	0.307	0.73	0.79	0.76	0.77	0.78	0.76	0.78
Oct. 19	High carbohydrate* Oct. 17, 18	500 grams beef	0.356	0.92	0.87	0.86	0.87	0.87		0.88
Oct. 20	High carbohydrate* Oct. 17, 18, 19	500 grams beef	0.318	0.92	0.85	0.89	0.87	0.88		0.88
Oct. 21	Carbohydrate diet Oct. 20 (55 grams cracker meal)	50 grams glucose	0.166		1.03	1.00	1.01	1.01		1.00
Oct. 22	25 grams glucose; 100 grams beef, Oct. 21	25 grams glucose; 100 grams beef	0.228		0.94	0.91	0.93	0.92		0.90
Oct. 23	25 grams glucose; 100 grams beef, Oct. 22	25 grams glucose; 100 grams beef	0.224		0.89	0.87	0.88	0.89		0.89
Oct. 24	25 grams glucose; 100 grams beef, Oct. 23	25 grams glucose	0.121		0.95	0.96	0.96	0.98		1.01
Oct. 27	High carbohydrate* Oct., 24, 25, 26	None	0.092		0.91	0.90	0.90	0.90		0.90

* 110 grams cracker meal, 10 grams glucose, and 40 grams beef heart.

† 40 grams lard and 40 grams beef heart.

‡ All determinations of nitrogen are the average of the experimental period, and consequently are not accurate indices, in protein feeding experiments, of the true protein metabolism.

On October 14 no food was given, but the effect of the high protein feeding was still manifest during the early hours, for the pre-exercise resting quotient was 0.76 and that of exercise 0.77, later dropping to 0.73 at rest (the N excretion was also still elevated). However, after another

TABLE 2

Energy exchange at rest and in exercise

DATE	EXPERIMENTAL DIET	RESTING METABOLISM (PER HOUR)	EXCESS METABOLISM (OVER BASAL)*	WORK†	CALORIES TO MOVE 1 KG. 1 METER	INCREASE OVER POST-ABSORPTIVE EXCESS	SPECIFIC DYNAMIC ACTION
1931		*calories*	*calories*	*kgm.*		*per cent*	*per cent*
Oct. 6	Basal	14.0	7.1	7,340	0.97		
Oct. 8		14.4	9.0	7,145	1.26		
Oct. 14		16.2	6.5	6,698	0.97		
Oct. 15		15.8	6.9	6,578	1.05		
Average		15.2	7.4	6,940	1.06 (1.00)‡		
Oct. 9	Beef, 500 grams	19.4	10.8	7,016	1.54		28
Oct. 12		21.2	10.0	6,726	1.49		39
Oct. 13		20.6	9.3	6,698	1.39		36
Oct. 16		20.8	9.8	6,698	1.46		37
Oct. 19		22.6	10.6	7,002	1.51		49
Oct. 20		23.0	12.1	7,298	1.66		51
Average		21.2	10.4	6,906	1.51	45 (51)	40
Oct. 21	Glucose, 50 grams	19.8	8.5	7,032	1.10		30
Oct. 24	Glucose, 25 grams	18.6	7.6	7,556	1.01		22
Average		19.2	8.1	7,294	1.06	0 (6)	26
Oct. 22	Glucose, 25 grams;	20.4	8.5	7,032	1.10		34
Oct. 23	beef, 100 grams	20.2	8.3	6,834	1.22		33
Average		20.3	8.4	6,933	1.16	12 (16)	34

* Not over resting metabolism of the experiment itself except in "basal" group.
† Loosely used to represent weight moved through distance horizontally.
‡ Excluding October 8.

day of starvation both the resting R. Q. and that of exercise had fallen to the normal fasting levels (0.73 and 0.72, respectively). After the experiment, on this day, a high fat diet was again given, and on the following day the post-absorptive quotient was still 0.73. Upon giving 500 grams of beef, the R. Q. jumped to 0.79 before exercise and 0.78 during exercise and recovery; falling later to 0.76 as the protein effect wore off.

From the above experiments, it seems evident that whereas on fat feeding or during starvation the animal used fat as a source of oxidative energy both at rest and during exercise, confirming previous observations (Rapport and Ralli, 1928) upon the ingestion of protein the animal not only oxidized protein during rest, but also during exercise, for the rise in quotient was large and unmistakably beyond any error in the method. The R. Q. being approximately the normal R. Q. of meat protein, we must conclude that both the sugar forming and non sugar-forming amino acids were oxidized.

In table 2 will be seen data on the energy exchange, which show that it required 45 per cent more energy to move 1 kilo 1 meter after 500 grams of meat had been fed than in the post-absorptive state, and there is no doubt, as originally shown by Rubner (1910) and confirmed by Anderson and Lusk (1917) and Rapport (1929), that the specific dynamic action of the protein was not abolished during the exercise. It is reasonable to suppose, though admittedly not absolutely proved, that in these experiments, the split-products of amino acid metabolism were oxidized for energy in exercise without having been first converted to glucose and fat. If this is true, it would throw doubt on theories which postulate that the specific dynamic action of protein is due to waste energy involved in the conversion to glucose.

Protein feeding. (*b*) *After carbohydrate diet.* This phase of the work requires a few words only. Reference to experiments 1, 10, 11, and 15 in table 1 will show that these experiments were not conclusive. In the first place we were unable to obtain an exclusive carbohydrate metabolism, the post absorptive R. Q. being never above 0.92. In the second place, in the presence of the carbohydrate plethora, it is of interest that we could not produce an exclusive protein metabolism on giving meat, the resting quotient not going below 0.87. It is difficult to say what percentage of protein metabolism this represents; if protein were substituted for fat metabolism, the maximum protein metabolism would have been about 60 per cent, but it would not be safe to make the assumption that this is what actually happened. Such change as occurred in the resting quotient, however, was reflected in the exercise quotient, and, as far as they go, the experiments were confirmatory of those after fat ingestion.

Mixed protein and carbohydrate feeding. We wished to see whether the type of food used in exercise would be different if carbohydrate alone were given or a mixture of carbohydrate and protein. On October 21, 50 grams of glucose were administered with resulting resting and exercise quotients of 1.01 and 1.00 respectively. On the following day a mixture of 25 grams of glucose and 100 grams of beef heart, having a theoretical respiratory quotient of 0.90, was given. The average resting R. Q. was 0.93; the excess R. Q. of exercise and recovery 0.90. The next day the experiment

was repeated, and again the excess quotient of exercise (0.89) was practically the theoretical quotient of the mixture, although 25 grams of glucose alone contained more than enough energy to take care of the exercise. Giving this amount of glucose alone on the next day resulted in an excess quotient of 1.01, showing exclusive use of sugar in the exercise.

These experiments showed that in the presence of a plethora of both sugar and protein each could be used in exercise without apparent preference for either.

Extending the conclusions of previous work, it appears that in muscular exercise, the oxidative energy can be supplied not only by carbohydrate and fat, but by protein as well, and apparently quite as readily by the latter as by the other two major foodstuffs.

CONCLUSION

Protein is a normal source of oxidative energy for muscular exercise, and its non-nitrogenous split products are as readily available for this purpose as are carbohydrate and fat.

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SYMPATHIN AND THE HEPATIC SYMPATHOMIMETIC HORMONE IN THE DOG

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Previous work on sympathin (Newton, Zwemer and Cannon, 1931; Cannon and Bacq, 1931; Rosenblueth and Schlossberg, 1931; Rosenblueth and Cannon, 1932) and on the adrenin-like substance liberated from the liver by stimulation of its sympathetic supply (Cannon and Uridil, 1921; Cannon and Griffith, 1922; Rosenblueth and Cannon, 1932) has been performed mainly on cats. The only exception is Bacq's (1931) work on the iris of the rabbit. It was considered of interest to learn whether similar phenomena would be presented by other species, thus giving a more general character to the conclusions derived. After some trials on the heart and blood pressure of the rabbit the dog was chosen because of greater ease in applying the techniques involved.

METHOD. The responses of the denervated heart were studied either by using the method employed by Cannon and Bacq (loc. cit.) or by placing the animal under light dial anesthesia. The changes of blood pressure were investigated with the preparation described by Rosenblueth (1932).

The heart was sensitized by denervation at least 5 days before. The sensitization of the blood-pressure response by cocaine was generally increased by removal of the stellate, semilunar, and superior and inferior mesenteric ganglia, again at least 5 days before.

The adrenals were commonly ligated or otherwise inactivated, either acutely or several days before the experiment.

Heart rates and blood pressures were recorded through femoral or carotid arterial cannulae connected to a mercury manometer. Stimulation of the lower abdominal sympathetic chains (l.a.s.) at about the level of L3 and of the nerves surrounding the duodeno-hepatic artery (h.n.), after both groups of nerves had been centrally crushed or cut, was effected by shielded electrodes in the secondary circuit of a Harvard inductorium. Tetanizing frequencies were used.

RESULTS. The responses of the above indicators differ only quantita-

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tively when either the l.a.s. or the h.n. are stimulated for 30 to 60 seconds. The responses are greater in the second case.

There is an increase of the rate of the denervated heart, which begins to be noticeable about one minute after the start of stimulation, though sooner for the h.n. than for the l.a.s. It reaches its maximum about 3 minutes later and lasts between 5 and 10 minutes. The increases observed in these experiments ranged from 10 to 30 beats per minute (approximately from 6 to 20 per cent). They could be repeatedly obtained. The highest rise in the rate of the heart coincided in time with the peak of the blood-pressure changes.

When the l.a.s. is stimulated there is an initial rise of blood pressure (see fig. 1), due undoubtedly to vasoconstriction. This persists during stimulation, producing a plateau. About one minute later, usually after the end of the stimulation, a second rise appears, bringing the blood pressure to a higher level. Finally there is a gradual return to normal. Sometimes both rises blend into a single curve, but there is invariably, for about two minutes after the stimulation has ceased, a continuing increase which cannot be accounted for by the vasoconstrictor effects of the nervous stimulation.

Stimulation of the h.n. yields similar but more marked effects. These persist when the hepatic artery is clamped.

If the circulation to the hind part of the body is occluded by pulling a thread passed around the aorta and the cava below the renal vessels there is a rise of blood pressure (see fig. 2-A). When the thread is released there is a sharp fall, followed by a slow return to the original level. If the l.a.s. are stimulated while the thread is being pulled (see fig. 2-B) the rise during the occlusion is followed by a fall on release, but the pressure does not go below the base line. There then occurs a new rise, usually higher than that resulting from pulling the thread, which, again, cannot be accounted for by the constrictor effects of the stimulation.

Emotional excitation of the sympathetic in the unanesthetized animal (Newton, Zwemer and Cannon, loc. cit.) increases also the rate of the denervated heart even when all nervous connections to the liver and adrenals have been removed. Complete sympathectomy abolishes this response of the heart. Similar results were observed by us in the dog.

SUMMARY

The responses of the denervated heart and of blood pressure to peripheral stimulation of the hepatic nerves and of the lower abdominal sympathetic chains were studied in the dog.

The results obtained (see figs. 1 and 2) are strictly comparable to those found in the cat in previous papers published from this laboratory.

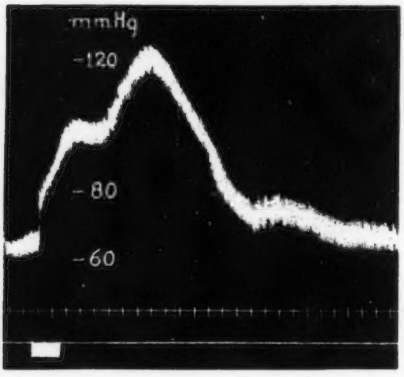

Fig. 1. Blood pressure response on one minute peripheral stimulation of the sectioned lower abdominal sympathetic chains at L3. Dial, 0.25 cc. per k. intravenously. Cocaine, 8 mgm. per k. intravenously. Vagi cut. Stellate ganglia and upper thoracic sympathetic chains removed 6 days previously. Spinal cord transected at D2 and adrenals inactivated the day preceding the experiment. Time intervals, 30 seconds.

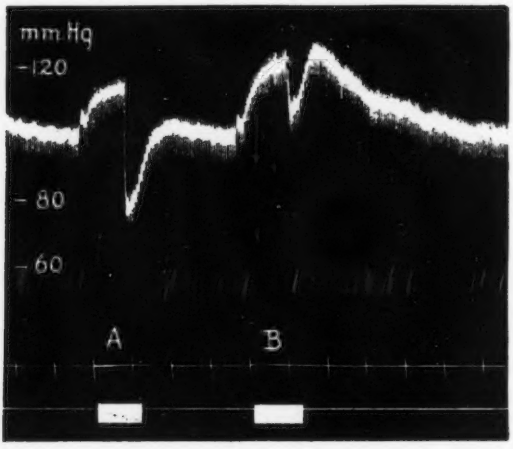

Fig. 2. Stellate, semilunar, and superior and inferior mesenteric ganglia removed 10 days before. Dial, 0.7 cc. per k. intraperitoneally. Vagi cut. Adrenals ligated. Heavy thread passed under the aorta and the vena cava immediately below the renal vessels. Time intervals, 1 minute.

A. Effects of occluding the circulation to the hind part of the animal 1 minute.

B. Similar occlusion with simultaneous excitation of the lower abdominal sympathetic chains. See text for interpretation of records.

Sympathin and the sympathomimetic substance liberated from the liver are, therefore, not limited to the feline species.

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STUDIES ON THE REGULATION OF WATER INTAKE

I. The Effect of Extirpation of the Salivary Glands on the Water Intake of Dogs while Panting

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The close correlation between appearance of thirst and a marked reduction in the salivary flow observed by Cannon (1918) led him to conclude that thirst is really what the ordinary individual thinks it is, namely, a dryness of the mouth, and that it is brought about when the salivary glands are prevented from discharging sufficient fluid with which to moisten adequately the buccal mucosa. Montgomery (1931a and b), in experiments on dogs deprived of their salivary glands, or studied in relation to the effects of drugs (pilocarpine and atropine) on the water intake, has obtained results which tend to discredit this explanation of the thirst sensation and to minimize the importance of the salivary glands in relation to the water intake. She has offered no alternative explanation. The results are of a negative character.

The problem of the importance of the salivary glands in relation to thirst raises two crucial questions: 1. Can thirst, when caused by water deprivation, be alleviated by merely wetting the mouth? 2. Does thirst appear in the absence of bodily dehydration when mouth and throat become dry?

1. Can thirst, when caused by water deprivation, be alleviated by merely wetting the mouth? Common testimony indicates that there is an urgent desire for water before the subject is aware of general functional disturbances, due to need for it, and this is borne out by the fact that the subject may obtain relief from measures which do not increase the water content of the body. "Commonly the condition is alleviated by a moderate quantity of water; sometimes fruit acids and other sapid substances exciting flows of saliva are requisite for relief; and in the practical life of the range a pebble or nail carried in the mouth is often efficacious . . . ." (McGee, 1906). "When water is scant . . . . it may be economized by a method well known in all arid regions—that of alleviating local dryness of the buccal and other membranes by sipping and sniffing a few drops at a time, and allowing the general condition to take care of itself. Many vaqueros and prospectors become artists in mouth moistening and carry

canteens only for this purpose (depending on lavish draughts at camp to supply the general needs of the system) . . . . " (ibid.). From McGee's (1906) personal observations one cannot fail to be impressed with the fact that the thirsty man's chief concern is to moisten his mouth and be relieved of the tormenting dryness in his mouth and respiratory passages.

Some years ago Pack (1923) showed that the quantity of water drunk in one hour by rabbits which had been deprived of water for seven days was greatly reduced by an injection of pilocarpine (0.5 cc. of 1 per cent solution per kilogram body weight) given shortly before water was offered the animals. Pack concluded from these observations "that pilocarpine . . . . relieves thirst by stimulating the flow of saliva." His experiments have been repeated in this laboratory (Gregersen and Bloomberg, unpublished) and the observations confirmed, but it was noticed that in every case the injected rabbits were more or less prostrated for some time after the drug had been given. Therefore, it cannot be said with certainty that these rabbits drank less than the controls solely because of an increased flow of saliva. It seems probable that the effect of the pilocarpine upon their general well-being caused them to drink less.

Montgomery (1931b) found that "injections of pilocarpin, after a two day thirst period, did not diminish the water intake of normal dogs as compared with that of dogs which had been deprived of their salivary glands," and, except in one case, the dogs which received pilocarpine drank more than the dogs which had not. These observations indicate that pilocarpine fails to relieve thirst by promoting a free flow of saliva and, therefore, disagree with Pack's experiments. We have repeatedly observed that any dose of pilocarpine which induces abundant flow of saliva also has a depressant effect on dogs. Indeed, Montgomery (1931b) frankly states that in several of her experiments the drug had an emetic action. If vomiting is accompanied by as much discomfort and malaise in the dog as in man, one can hardly regard a pilocarpinized animal as being in a favorable condition for showing whether or not an increased salivary flow can alleviate thirst. It would be advisable to study the relation between salivary flow and thirst without recourse to experimental procedures with such general and ill-understood bodily effects.

The question of whether or not thirst can be alleviated by merely wetting the mouth is therefore left unanswered by experiments involving the use of pilocarpine for exciting a flow of saliva, and we are left to draw conclusions from common experience.

2. Does thirst appear in the absence of bodily dehydration when mouth and throat become dry? "Breathing hot air free from moisture, prolonged speaking or singing, the repeated chewing of desiccated food, the inhibitory influence of fear and anxiety on salivary secretion, have all been observed to result in dryness of the buccal and pharyngeal mucous membrane and in

attendant thirst" (Cannon, 1918). Whether or not this form of thirst is exactly the same quality of sensation as the thirst produced by water deprivation, it is at least evident that the end result is the same, namely, the desire to drink, and it shows that local dryness of the mouth unaccompanied by bodily need for water may lead to increased water intake.

Montgomery (1931a) attempted "to make a crucial test of the importance of the salivary secretion in the control of water ingestion by completely extirpating the salivary glands." This investigator reasoned that "if . . . . a diminished secretion of saliva leads to local dryness of the mouth, which in turn causes the animal to drink, a total elimination of the salivary secretion should produce an increase in the daily ingestion of water by the animal" (1931a).

After tying the salivary ducts in dogs, Bidder and Schmidt (1852) observed a remarkable decrease in the fluid wetting the mouth so that only at best was this sufficient for keeping the mucosa moist, when the mouth was held closed; " . . . . bei erleichtertem Luftzutritt . . . . ein wirkliches Trockenwerden" was scarcely prevented. The dogs had difficulty in swallowing not only dry food such as bread, but even raw meat. Thirst was greatly increased so that the dogs were always ready to drink. Unfortunately, Bidder and Schmidt gave no data on the water intake. In two dogs Fehr (1862) extirpated the salivary glands, including the infraorbitals. After the immediate effects of the operation had worn off, the only change noticed was that the dogs then drank somewhat more water "probably to facilitate chewing and swallowing of food." Montgomery (1931a), on the other hand, found that the average daily water intake of dogs kept under laboratory conditions was not increased after the complete removal of submaxillary, sublingual, parotid and infraorbital glands. She concluded from her observations, "it appears improbable that the salivary glands play a major rôle in the thirst mechanism."

But this conclusion does not necessarily follow from her data. Her observations were made under conditions where a small secretion into the buccal cavity may have been adequate for keeping the mouth and tongue moist enough to prevent increased thirst. After extirpation of the salivary glands the dogs still possessed other glands which could supply fluid for wetting the buccal cavity. The secretion must have been adequate to prevent drying of the mucosa *under the conditions of her experiments*, for she speaks of "the remarkably moist condition of the mucous membrane of the mouth which can exist in the entire absence of the salivary glands." This agrees with observations we have made. In one of Montgomery's dogs, no. 7, the secretion poured out by the glands of the buccal, pharyngeal and nasal mucosa was collected from an esophageal fistula. The secretion measured 8.9 grams per hour when the dog was quiet, 1 gram per hour when the dog was asleep. This, of course, does not include water carried

off by the expired air. Removal of the salivary glands has obviously not eliminated all mechanisms for keeping the mouth wet.

Montgomery's dogs, moreover, were well supplied with water and were given a moistened food (25 grams of water per 100 grams of food). The activity of the remaining secretory tissue in the buccal cavity was therefore not depressed by bodily dehydration. It is evident that, although the salivary glands supply a watery secretion, they cannot be regarded as the sole

TABLE 1

Showing the effect on water intake before and after exclusion of the salivary flow when dogs were exposed to a temperature of 40°C.

DOG NUMBER	WEIGHT	WATER SUPPLIED BEFORE EXPERIMENT	DURATION OF EXPOSURE	BEFORE OPERATION			AFTER OPERATION			CHANGE IN WATER INTAKE	AFTER / BEFORE
				Number of observations	Water taken (average)	Water taken	Number of observations	Water taken (average)	Water taken		
	kgm.		*hours*	*days*	*cc.*	*cc. per kgm.*	*days*	*cc.*	*cc. per kgm.*	*cc.*	
1	20.0	ad libitum	2	7	301	15.0	29	586	29.3	+285	1.95
2	15.0	ad libitum	2	9	447	30.0	19	417	27.8	−30	0.94
3	12.0	ad libitum	2	11	217	18.0	18	450	37.5	+233	2.07
4	13.5	ad libitum	2	9	231	17.0	23	518	38.4	+287	2.24
6	18–19	ad libitum	1	8	46	2.5	10	134	7.3	+88	2.9
	(18.5)	none for 6 hours	1	7	103	5.6	7	136	7.4	+33	1.32
		none for 18 hours	1	6	183	9.9	14	209	11.3	+26	1.14
7	13–15	ad libitum	1	8	195	13.9	12	287	20.5	+92	1.47
	(14.0)	none for 18 hours	1	5	350	25.0	17	513	36.6	+163	1.47
8	18.0	ad libitum	1	8	72.5	4.0	9	244	13.5	+171.5	3.36
		none for 18 hours	1	6	153	8.5	21	350	19.4	+197	2.29
9	21–19	ad libitum	1	8	135	6.8	9	72	3.6	−63	0.53
	(20.0)	none for 6 hours	1	7	127	6.4	9	105	5.3	−22	0.83
		none for 18 hours	1	6	263	13.2	15	190	8.5	−73	0.72

Dog 5 in this series was a young animal that died of distemper.

source of fluid wetting the mouth. On the other hand, the fact that *under favorable circumstances* the mucous glands may moisten the mouth sufficiently to obviate thirst does not rule out the importance of the salivary glands when there is greater need for fluid than the other glands of the mouth can supply.

To find what rôle the salivary glands actually play in the thirst mechanism, we believe that the effect of their extirpation upon water intake

should be tested under experimental conditions which lead to drying of the buccal mucosa. This can be accomplished by taking advantage of a special mechanism in the dog for lowering the body temperature. When exposed to a warm atmosphere the dog begins to pant. The mechanism is reflex and mediated by cutaneous sensory nerves (Sihler, 1879). The panting is accompanied by a flow of thin watery saliva (Welikanoff, 1928) which in this case has been definitely shown (Gregersen, 1931) to arise from dryness of the mouth and tongue and which helps to keep the mouth moist. In the absence of salivary glands, dryness of the mouth would be intensified since it can no longer be mitigated by a reflex secretion of

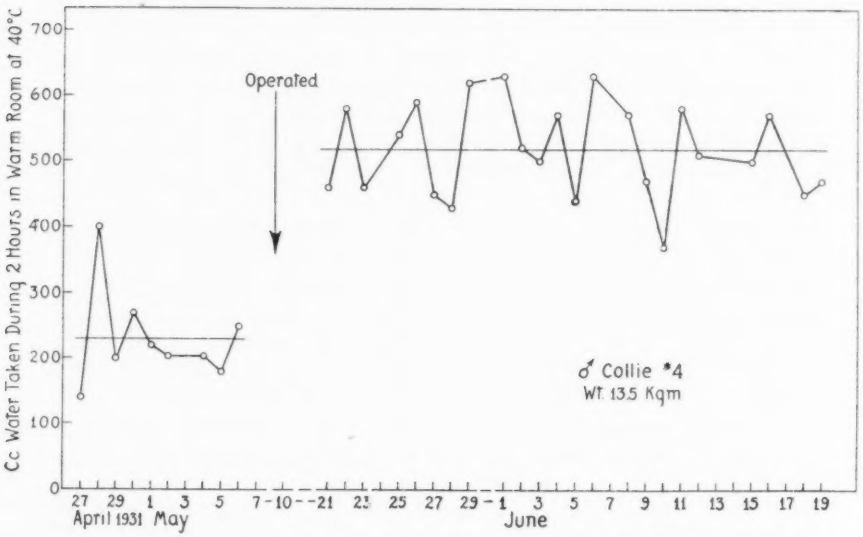

Fig. 1. Daily observations on dog 4, showing the amount of water taken during 2 hours in the warm room (40°C.) before and after exclusion of the salivary flow.

saliva. If thirst is caused by a dry mouth, the dog should drink more than normally.

EXPERIMENTS. We have attempted to investigate this question by measuring the water intake of dogs during periods of panting before and after exclusion of the salivary flow. Four dogs were used in the first series. They were kept in a warm room (40°C). for 2 hours at approximately the same time each day, and the water drunk during the 2 hours was measured. At this temperature the panting was usually continuous, although dogs do not all respond equally well to a warm atmosphere. After readings had been made during a control period lasting on the average 10 days, the

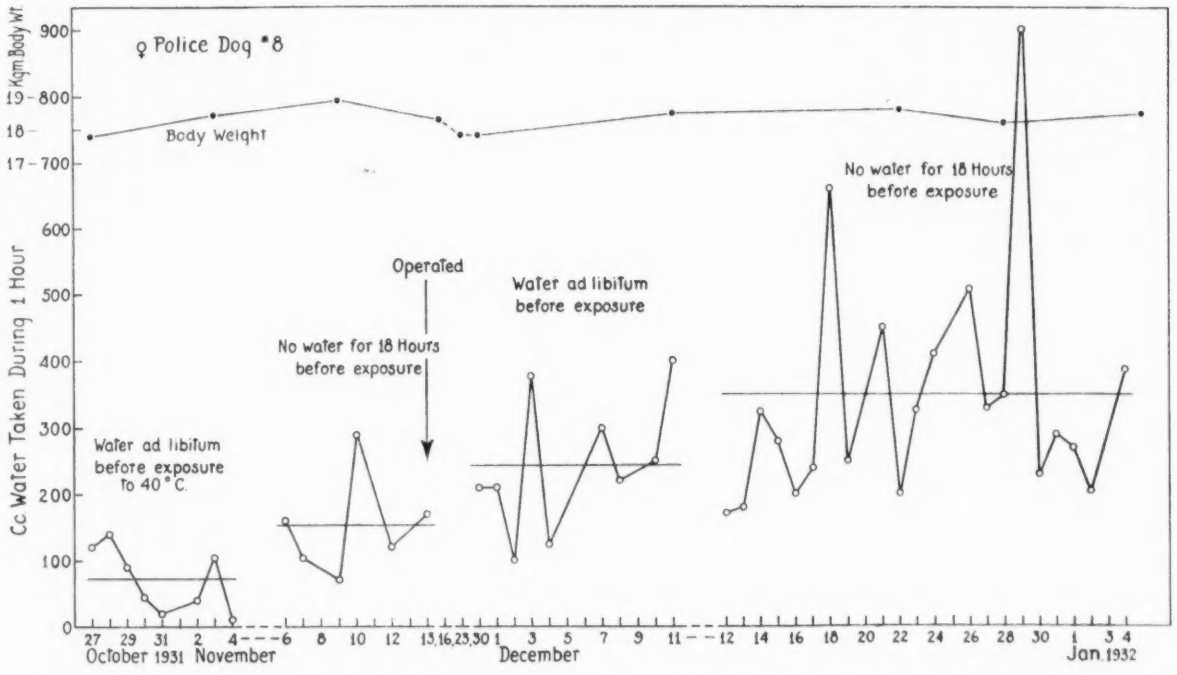

Fig. 2. Daily observations on dog 8, showing the effect of exclusion of the salivary flow on the water intake during 1 hour in the warm room (40°C.) when the dog had water *ad libitum* before entering and also when it had none for 18 hours before.

parotid ducts were tied and cut between ligatures, the submaxillary, sublingual, and infraorbital glands were extirpated, all in the same operation and with aseptic precautions. Recovery was complete in about 10 days, except in the case of dog 2 which required 20 days. The dogs were again placed in the warm room for 2 hours daily and the water intake during the 2 hours measured. Averaged results are shown in table 1 and the daily observations on one of the dogs (4) in figure 1.

In the second series (dogs 6, 7, 8 and 9), the daily exposure to 40°C. was limited to 1 hour. Tests were made not only when the dogs had been given water *ad libitum* up to the time of entering the warm room, but also when they had been deprived of water for 6 hours and for 18 hours before each test. The averaged results for these animals are also included in table 1 with those of the first series. The daily observations on one of the dogs (8) are shown in figure 2.

EXPERIMENTAL RESULTS. With the exception of dog 2, which was an old dog in rather poor condition, the animals of the first series drank approximately twice as much water during 2 hours in the warm room after the operation as before. When calculated in terms of centimeters of water taken per k. body weight, the water intake of dog 2 was much higher than in the others during the control period,—nearly as high as for the other dogs after the operation. It may be that the salivary flow of dog 2 was already deficient before the glands were removed. Autopsy failed to show anything to account for this exceptional result.

In the second series of animals we again find one dog (9) which failed to show an increase in the water intake after the operation. This dog suffered for over two weeks from an extensive subcutaneous necrosis in the groin and abdominal region, due to an infection. The lymph nodes in the groin were greatly enlarged, and each day for some time necrotic material was discharged through a hole in the skin. In two weeks the dog lost 3 k. in body weight. These complications, we believe, are sufficient reason for excluding the results obtained on this dog.

From the second series it may be seen that water deprivation for 6 hours or for 18 hours made surprisingly little difference in the water intake in the warm room. The animals were fed immediately after the daily tests and were given water for some hours after eating. In the following paper it will be shown that dogs (with or without salivary glands) drink very little during 24 hours except in the postprandial period. We can therefore understand why depriving the animals of water, except during 2 to 5 hours after feeding, causes an insignificant reduction in the 24-hour intake.

From the results of both series of experiments it appears that 6 of the 8 dogs drank more water in the warm room after the operation. This was true whether the dogs were deprived of water for some hours before the tests or were given water *ad libitum*. The last column in table 1 shows the

ratio of water intake after the operation to water intake before it, under the same experimental conditions.

As a rule, the dogs in the second series did not pant as vigorously or continuously in the warm room as the first series of animals, perhaps due partly to the shorter time of exposure. Dogs differ, however, in their response to heat and it may be that more marked results could be obtained if animals were chosen which pant consistently when exposed to high temperatures.

Autopsies on dogs 1, 2, 7 and 9 showed that the operations on them had been successful.

The same diet, consisting of ground cooked horse meat mixed with "Old Trusty All Terrier Food," was given throughout the experiments. The dogs took this mixture eagerly, although they were seen to eat more slowly after the operation.

SUMMARY AND CONCLUSION

The amount of water taken by dogs during 1 or 2 hours of panting is increased after extirpation of the submaxillary, sublingual and infra-orbital glands and tying the parotid ducts. Therefore, under conditions which make the mouth dry, a deficient salivary flow causes thirst and increased water intake even in the absence of bodily dehydration (see table 1, and figs. 1 and 2).

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STUDIES ON THE REGULATION OF WATER INTAKE

II. Conditions Affecting the Daily Water Intake of Dogs as Registered Continuously by a Potometer

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In carrying out a series of experiments on the rôle of the salivary glands in relation to the water intake of dogs (Gregersen and Cannon, 1932), I felt that records of their drinking habits were essential in order to be sure that the drinking was not affected by factors other than those we were deliberately imposing upon them. This led to the construction of a simple apparatus for obtaining continuous 24-hour records of the water intake. A study of these records sheds new light on the relation between digestion and drinking.

The potometer. A diagram of the essential parts of the apparatus may be found in figure 1. A reservoir, *R*, consisting of an airtight copper can of 1.5 liters capacity, is suspended from a coiled spring which shortens about 1 cm. for each 100 cc. of water taken from the can. It is connected by a rubber tube, *T*, supported by a wooden splint, to a short brass pipe, *P*, soldered to the side of a drinking pan, *DP*. Fluid leaves the reservoir whenever the water level in the pan falls below the outlet of the brass pipe, and this is registered by the shortening of the coiled spring. A writing point attached to the lower end of the spring records the emptying of the reservoir on a smoked drum which turns once in 12 hours. Removal of less than 25 cc. of water from the drinking pan is not recorded on the drum; therefore the record may not show the actual number of times water is taken.

The potometer is calibrated by pipetting 100 cc. at a time from the drinking pan and recording on the smoked drum the shortening of the spring. The graduated scale thus obtained enables one to read directly on the records the quantity of water taken by the dog over any given period of time. The calibration shows that the change in length of the spring upon decreasing the load 100 grams is about the same when the reservoir is nearly empty as when it is full. Therefore, no correction for changes in elasticity of the spring is necessary throughout the range of loads used here.

Correction for evaporation was not deemed essential since it amounted to less than 15 cc. per day.

The kymograph used was an old type which turns from left to right; therefore, in order to make the records read in the usual fashion they have been reversed. Also they have been turned upside down. Consequently, a downward stroke on the record signifies emptying of the reservoir.

EXPERIMENTS AND RESULTS. *Drinking records on normal dogs.* The drinking records shown in figure 2 are typical of those obtained on all normal dogs kept in cages in the laboratory. They live an inactive life, since their cages afford only limited space for moving about. Once a day

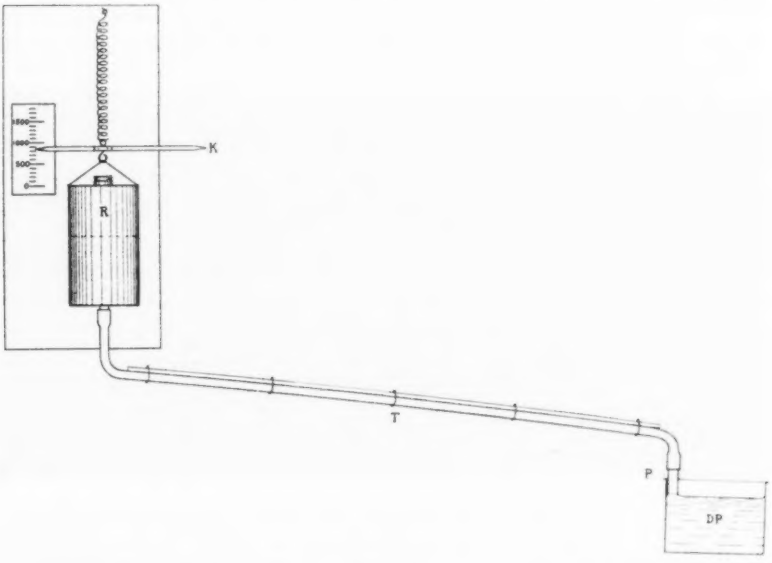

Fig. 1. The potometer. When the dog drinks from the pan *D P*, and the water level falls below the brass pipe *P*, air bubbles pass up the rubber tube *T* and release water from the reservoir *R*. The quantity of water taken is shown on the scale and is registered on a smoked drum by the writing point *K*.

they are fed a meal consisting of ground cooked horse meat mixed in about equal parts with "Old Trusty All Terrier Food," a crushed cracker.

The dogs all drink when they have eaten, rarely before the meal is finished, taking water from time to time during 2 to 5 hours after the meal; they drink very little until the next day when they are fed again (see records of "Mike" and dog 12, fig. 2). The drinking is evidently not a matter of habit associated with some particular time of day, for it always follows upon feeding no matter what time the food may be given (cf. records of dog 12 and "Mike," fig. 2). Moreover, the impulse to drink does not

arise solely, if at all, from the fact that the food may be dry and therefore hard to swallow, because the period of water intake extends over several hours after meals.

This increased desire for water postprandially, or "soif digestive,"(Longet, 1868; Binet, 1931) naturally raises the question of what happens to the drinking when no food is given.

Drinking records on fasting dogs. Figure 3 shows the effect of fasting upon drinking. Number 22, a normal police dog of 18 k., drank only about 100 cc. in 2 days when food was withheld (see record, fig. 3, March 10 to 12). On March 12 this animal was fed and within 15 minutes it took its first drink; it consumed 600 to 700 cc. in 4 hours and a total of about

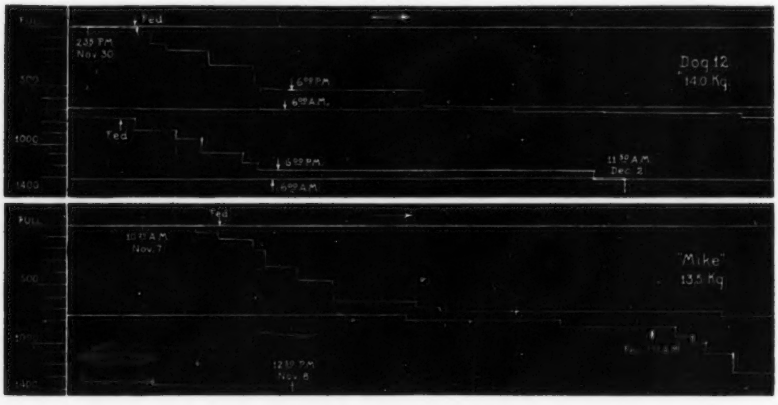

Fig. 2. Normal drinking records on dog 12 and "Mike," showing that most of the water drunk during 24 hours is taken within a few hours after the dogs are fed, no matter when the food may be given. In these and other drinking records the length of the record covers a period of 12 hours.

1000 cc. in 24 hours. "Mike," a terrier of 13.5 k., with double submaxillary fistulae of long standing, showed the same result less strikingly. During 48 hours of fasting, the dog drank in all 700 cc. On the third day he drank, within 5 hours after being fed, 750 cc.

These results are in agreement with observations made by Kleitman (1927). He found that starving dogs drink about 100 to 300 cc. of water per day, while the normal water intake, including water in food, is about 500 to 700 cc. per day. Kleitman (p. 339) suggests, merely as a possibility, that "the decrease in total metabolism, entailing a diminution in excretion of nitrogenous waste products and other crystalloids by the kidneys, with consequent smaller volume of urine, would naturally call for a smaller water intake, but whether this can be held responsible for the

decrease of 65 to 80 per cent in the total water intake actually observed during starvation remains to be proven." My records do not support such an explanation. If water were required mainly for "excretion of nitrogenous waste products and other crystalloids by the kidney," one would not expect to find it taken so promptly after the meal.

A more plausible explanation of postprandial thirst is found in the immediate processes of digestion. Bidder and Schmidt (1852) estimated that a dog of 16 k. secreted more than 1.6 liters of gastric juice in 24 hours, i.e., a volume of fluid equal to the total blood volume of the animal. Pawlow (1889) collected 20 cc. of gastric juice in 5 minutes (200–300 cc. per hour) from a dog's gastric fistula by sham feeding. Wolfsberg (1914) fed a dog 200 grams of raw meat and obtained as a result 535 cc. of gastric juice in 3.5 hours. Dragstedt and Ellis (1930) have found that dogs with isolated stomachs secrete 1000 to 2600 cc. of gastric juice in 24 hours.

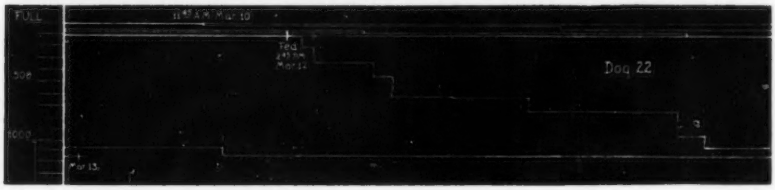

Fig. 3. Showing the effect of fasting upon the drinking of a normal dog (22). No food was given on March 10 and 11. From 11:45 a.m. March 10 until 2:45 p.m. March 12, when the animal was fed again, it drank only about 100 cc. Within five hours after the meal it drank more than 600 cc.

According to Mathews (1930) the duodenum of the dog secretes large quantities of alkaline juice; in one case 50 cc. were obtained in 2 hours from a dog weighing 5 k. In man it has been estimated that approximately 7.5 to 10.0 liters of fluid are daily secreted into the gastro-intestinal tract (Rowntree, 1922). The volume of the digestive secretions is evidently so large that their discharge may lead to a considerable decrease in the volume of the body fluids. Indeed, if in the dog reabsorption of the gastric juice is prevented by pyloric obstruction (Gamble and Ross, 1925), the body is rapidly dehydrated and the animal dies in a few days. These facts suggest that an explanation of the increased water intake after eating is to be found in the rapid secretion of digestive juices.

If the secretion of digestive juices is responsible for the postprandial thirst, it follows that the desire for water should disappear when, in the course of digestion, the secreted fluid has been largely reabsorbed, even though no water be taken after the meal. To test this proposition, dogs were fed but given no water until several hours later. The absence of

water during the meal did not prevent the animals from eating, as usual, all the food given.

Considerable variations in the results are found but in general the quantity of water taken in the 24-hour period is much reduced if the giving of water is delayed for several hours after feeding. A striking example from dog 20 is shown in figure 4. Two normal results were obtained on April 13

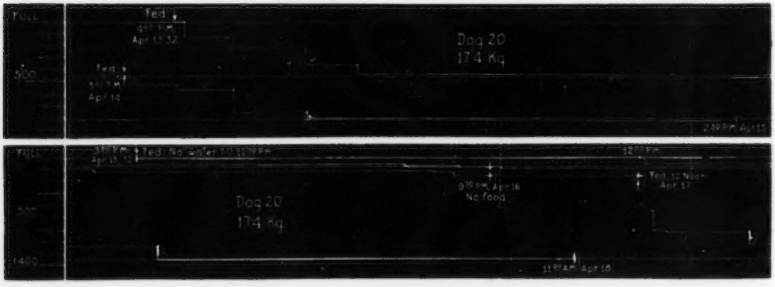

Fig. 4. Showing the drinking records obtained on dog 20 when *water was not given until several hours after feeding.* Normal postprandial records with water available were obtained on April 13 and 14. On April 15 food was given at 3:30 p.m. but no water until 12 midnight, i.e., 8.5 hours later. During the following 36 hours the dog drank only about 100 cc. Within 4 hours after being fed again (at noon, April 17) he drank about 700 cc.

Fig. 5. Drinking records on dog 6 in which the parotid ducts were tied and the submaxillary, sublingual and infraorbital glands extirpated in October, 1931. Normal records (Feb. 4 and 5) show that most of the water is taken shortly after eating, and in spite of absence of salivary flow little is drunk between meals.

and 14. On April 15 the dog was fed at 3:30 p.m. When offered water at 12 midnight he took only a small amount, and during the next 36 hours (without food) he drank only about 100 cc. When fed at noon April 17, he drank a large amount of water (about 700 cc.) within 4 hours, as usual after eating. These and other observations (Gregersen, 1932) indicate that postprandial thirst is due to temporary dehydration resulting from the rapid accumulation of secretions in the digestive tract after food is taken.

Drinking records from dogs after exclusion of the salivary flow. In figure 5 are shown normal drinking records from dog 6 in which the parotid ducts were tied and cut between ligatures, and the submaxillary, sublingual and infraorbital glands extirpated November 24, 1931. Normally this dog drinks 500 to 700 cc. of water per day. From the records (Feb. 4 and 5) it is clear that most of this water is drunk within a few hours after food is taken. In spite of the absence of the salivary glands the dog, when not required to pant, drinks no more than normal dogs during the time between meals.

In reconsidering the purpose for which this work was begun we see that the time of day during which Gregersen and Cannon (1932) made observations on the water intake of dogs in the warm room was a period during which normal and operated dogs alike rarely drink any water. It is therefore certain that they drank because they were exposed to heat and the consequent panting made them thirsty.

Records of several different animals have purposely been included to show that the phenomena observed are not peculiarities of one particular dog.

SUMMARY AND CONCLUSIONS

1. An instrument (the potometer) is described whereby the drinking of water is recorded graphically and quantitatively (see fig. 1).

2. Graphic records of the 24-hour water intake of dogs reveal that almost all the water is taken within 2 to 5 hours after feeding, regardless of the time food is given (see figs. 2, 3 and 4).

3. Fasting usually reduces the 24-hour intake to one-fourth normal or less (see fig. 3).

4. If the giving of water is delayed for several hours after feeding, the 24-hour intake is ordinarily much less than when water is given *ad libitum* throughout the postprandial period (see fig. 4). The postprandial thirst is therefore, in part, temporary. Reasons are given for attributing it to the withdrawal of water from the body for the secretion of digestive juices.

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ON THE MECHANISM OF PARATHYROID HORMONE ACTION

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It is now generally conceded that the increase in serum calcium and urine calcium which follows the injection of a potent parathyroid extract into a susceptible animal takes place principally at the expense of the calcium stores in the bones. The manner in which these stores are mobilized remains uncertain; three theories have been more or less explicitly put forward. It is suggested by Albright and co-workers (1930, 1932) that the parathyroid hormone lowers the renal threshold for inorganic phosphate, so that the plasma phosphate decreases and the plasma calcium is enabled to rise, the assumption being made that the plasma inorganic phosphate and calcium bear an inverse relation to one another, perhaps because the plasma behaves as if in equilibrium with solid tricalcium phosphate. The second theory has been expressed in different terms by different investigators, the central idea being that the administration of parathyroid hormone makes the plasma a better solvent for the calcium compounds of bone; for example, Greenwald (1926) suggests that there circulates in the plasma an organic substance, X, which is identical with the parathyroid hormone or is formed under its influence, and which unites with calcium ions to form an undissociated compound; so that the administration of parathyroid hormone directly or indirectly increases X, thus reducing the concentration of calcium ions in the plasma and permitting the liberation of more calcium ions from the bone; the suggestion that the plasma behaves as if in equilibrium with solid tricalcium phosphate is an integral part of the theory. The third view, which has not been thoroughly discussed from a physiological viewpoint, is that the parathyroid hormone directly stimulates cellular elements in the bone to increased osteoclastic activity. The object of this paper is to report experiments which are regarded as unfavorable to the first and second theories and hence as supporting the last.

METHODS. The experiments were carried out on unanesthetized dogs of about 20 kgm. weight, which were in most instances fasted for 18 hours before the experiment. Serum calcium was determined by the method of Clark and Collip (1925), serum inorganic phosphate by that of Fiske and Subbarow (1925), plasma pH by the colorimetric method of Cullen

(1922) with the aid of the angle centrifuge, and hemoglobin by means of the Newcomer plate.

EXPERIMENTS AND RESULTS. If the theory of Albright and his co-workers is applicable to dogs, then a rise in serum calcium produced by the injection of parathyroid extract should always be preceded or accompanied by a fall in serum inorganic phosphate. But in fact when parathyroid extract ("Parathormone" Lilly) is injected *intravenously* no such fall occurs, although the serum calcium displays the customary increase. In the two experiments illustrated in figure 1, the total serum inorganic phosphate increased slightly in one case and remained constant in the other. Since it might be urged, however, that not the total inorganic phosphate

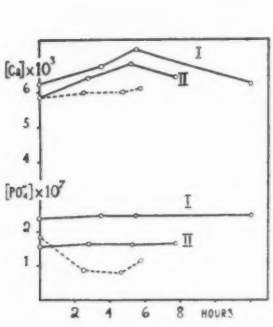

Fig. 1

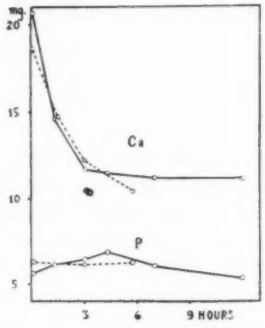

Fig. 2

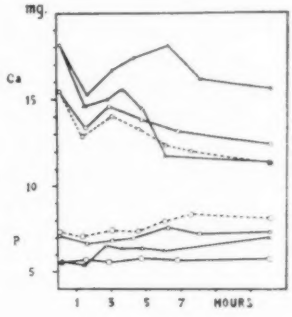

Fig. 3

Fig. 1. Serum calcium and serum $PO_4^{\equiv}$ ion concentration after the intravenous injection of parathyroid hormone (continuous lines) and after the administration of glucose and insulin (dotted line).

Fig. 2. Serum calcium and serum inorganic phosphate, in milligram per cent, after the intravenous injection of one gram of calcium chloride.

Fig. 3. Serum calcium and serum inorganic phosphate after the simultaneous intravenous injection of one gram of calcium chloride and 100 units of parathyroid hormone.

but only the ion species $PO_4^{\equiv}$ is of importance (in relation to the solubility product of tricalcium phosphate), the plasma pH was followed and the concentration of this species of ion calculated by means of the equation and constants of Sendroy and Hastings (1927). The continuous lines in figure 1 represent the course of two experiments of this type, in which 200 units of parathyroid hormone were administered intravenously. It is clear that the calcium, which is expressed in milli-equivalents per liter of serum, increases markedly in both cases, though, as Allardyce (1931) noted, the effect is more rapid, more transient, and less striking than after subcutaneous or intramuscular injection of the extract. On the other hand

the concentration of $PO_4^{=}$ ions, calculated from the total serum inorganic phosphate and the plasma pH and expressed in milliequivalents multiplied by 10^4, remains strikingly constant. Parathyroid extract does not appear to produce consistent changes in plasma pH. In the same figure the dotted lines show the effects of administering 40 clinical units of insulin subcutaneously and 50 grams of glucose orally to the dog used in the first parathyroid experiment. In this case there is a very great fall in total inorganic phosphate and in $PO_4^{=}$ ions (the pH being little affected) and only an insignificant rise in calcium, not outside the limits of accuracy of the method of determination. The failure of the calcium to rise is not due to dilution of the blood, since the serum proteins and the hemoglobin decreased only trivially during the experiment. It appears then that in the dog it is possible to raise the serum calcium by injections of parathyroid extract, without lowering the serum inorganic phosphate or $PO_4^{=}$ ions, and on the other hand it is possible to reduce the total phosphate and $PO_4^{=}$ concentration greatly, by the administration of glucose and insulin, while only slightly increasing the serum calcium. It is therefore impossible to suppose that the rise in serum calcium observed in the first case is secondary to a fall in the inorganic phosphate or any part thereof, and it also appears that in experiments of short duration there is little tendency for calcium and phosphate to display an inverse relationship.

The latter point is further illustrated in one of the two experiments shown in figure 2. These experiments serve merely to show the effect of intravenous injection (at the rate of 2.5 cc. per minute) of 20 cc. of 5 per cent calcium chloride solution. The serum calcium returns to normal levels in about five hours; the total serum inorganic phosphate in one case remains stationary and in the other increases transiently. It appears to be certain that the injection of phosphate lowers serum calcium, but the reverse is evidently not invariably true.

The experiments just discussed serve as controls for those shown in figure 3, in which the animals received calcium chloride solution as before, and simultaneously 100 units of parathyroid hormone intravenously. The object of this procedure was to test the second of the theories of parathyroid hormone action; it was felt that, if the rise in serum calcium which follows parathyroid administration is due to the plasma becoming a better solvent for the bone minerals, and if therefore the serum calcium peak represents the establishment of a new equilibrium, it should be possible by supplying extra calcium at the same time as the parathyroid extract to saturate the plasma immediately, so that the bones would not be attacked, and the peak of the serum calcium time-curve should appear immediately after the injection instead of some hours later. In other words, it would be anticipated on this theory that in such an experiment the serum calcium should decrease steadily from the beginning of the

experiment, but more slowly than in the experiments in figure 2 because of the presence of excess parathyroid hormone. This expectation is not realized; on the contrary, it is perfectly clear from the shape of the curves that there is no interaction between the injected calcium and the injected parathyroid hormone; the calcium curves of figure 3 may be imitated by superimposing those of figure 1 on those of figure 2. The decrease in serum calcium in the first two hours, while the injected calcium is disappearing from the blood stream and before the action of the parathyroid extract has become manifest, is not due to dilution of the blood, since the hemoglobin content remains practically constant. The control experiments show that the second rise is not due to the removal of blood for analysis. In one of the experiments, that shown by a dotted line, the calcium chloride solution and the parathyroid extract were mixed and incubated together for twelve hours before injection; there is no evidence that they formed a compound. It will be noted again that in these experiments the rise in serum calcium is not accompanied by a fall in inorganic phosphate.

Discussion. The experiments described show definitely that, in the dog, the rise in serum calcium after parathyroid injection is not secondary to any fall in phosphate; if the renal threshold for phosphate is affected, this change is not reflected in the composition of the blood. The fact that serum calcium can be slightly increased by the administration of glucose and insulin has been noted by many workers. The later experiments are unfavorable to the view that the parathyroid hormone increases the solvent power of the serum for the calcium salts of bone, though they are not irreconcilable with possible adaptations of this view. Hastings, Murray, and Sendroy (1927) showed that the power of serum to dissolve calcium salts *in vitro* was not lessened by thyroparathyroidectomy. Collip (1926) obtained double-peaked curves, similar to ours, after simultaneous injection of parathyroid hormone and calcium chloride; in his experiments the hormone was given subcutaneously, and the failure of the two curves to coalesce might have been ascribed to slow absorption; Inaba (1930) has also published similar curves, but the known unreliability of the rabbit in experiments of this type detracts from the decisiveness of his results in this particular argument. Our conclusion is, therefore, that the most acceptable theory of parathyroid hormone action is that it directly stimulates the osteoclastic process in bone. This view and its implications is more fully discussed by one of us (Thomson and Collip, 1932) in another place; it is sufficient to point out here that the histological changes seen in the bones of parathyroid-treated animals by Jaffé and Bodansky (1930), Bülbring (1931), and especially Selye (1932) suggest active destruction rather than passive solution of the bone mineral deposits.

SUMMARY

The rise in serum calcium which follows the injection of potent parathyroid extract, intravenously, in susceptible unanesthetized dogs, is not necessarily accompanied or preceded by a decrease in the total inorganic phosphate or the $PO_4^{=}$ ions of the serum. If such a decrease is produced by the administration of glucose and insulin, it leads only to insignificant increase in the serum calcium.

The rate of disappearance of injected calcium from the blood stream is not apparently affected by the simultaneous injection of parathyroid extract; the subsequent action of the extract in raising the serum calcium is not apparently modified by the injected calcium chloride solution; this finding is regarded as evidence against the view that the parathyroid hormone increases the solvent power of the plasma for the calcium compounds of bone.

The view that the parathyroid hormone actively stimulates the osteoclastic process in the bone receives support from these experiments.

We desire to express our thanks to Dr. J. B. Collip for his interest in this research, and to Dr. J. S. L. Browne for valuable assistance.

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THE EFFECTS OF THE GONADAL-STIMULATING HORMONE OF THE ANTERIOR PITUITARY ON THE VOLUNTARY ACTIVITY, THE AGE OF MATURITY AND THE SIZE OF THE LITTER IN IMMATURE FEMALE ALBINO RATS

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Earlier investigators have proven that in normal female rats there is an increased voluntary activity coinciding with the heat or oestrous period, which occurs on the average every fourth or fifth day (Long and Evans, 1922; Wang, 1923; Slonaker, 1925; Durant, 1924, 1925; Hitchcock, 1925, and Bugbee and Simond, 1926). It seemed that if it was the gonadal-stimulating hormone of the pituitary that produced these rhythmic heat periods in the adult female animal, then this same hormone, when injected into immature female rats, might produce an increased voluntary activity, or cause rhythmic variations of activity similar to that found in the normal matured animal. It has been experimentally proven (Smith and Engle, 1927; Zondek and Aschheim, 1927) that sexual maturity can be induced in immature animals (rats and mice) by means of transplanting anterior lobe pituitary gland either subcutaneously or intramuscularly. The number of transplants required to produce the effect depends upon the age of the rat and varies in individuals.

These investigations were carried out to determine the effects of the pituitary hormone on voluntary activity, age of puberty, and the size of the litters.

PROCEDURE. Immature female albino rats ranging from eighteen to twenty-two days of age were used as subjects for this experiment.

The technique was that employed by Smith (1927). Immature and mature rats of both sexes were used as donors of the pituitary tissue. While it has been shown by Engle (1929), and Evans and Simpson (1929, 1929a), that the potency of pituitary substances varies with age, sex and physiological conditions, no account of this fact was taken in this work. In all cases sufficient material was injected to produce the desired effects regardless of the potency of the transplants.

The recipients were divided into four different classes and each of these, except group I, were divided into subdivision A and subdivision B. All the animals in subdivision A were the progeny of mothers that matured

normally; those in subdivision B were the progeny of precocious sexually matured mothers. The first class comprised thirty-two animals that were injected with two glands per day for two successive days. In the second class (subdivision A—sixty animals, subdivision B—sixty animals), two glands were injected each day for two successive days followed by a second injection of three glands five days after the last injection and this followed by a third injection of three glands four days after the second injection. In the third class (subdivision A—seventy animals, subdivision B—eighty animals), each animal received daily, until the vaginal canal opened, 1 cc. of an extract D "which contained a considerable amount of the gland material in the form of an emulsion." Animals in the fourth class (subdivision A—one hundred and ten animals, subdivision B—seventy animals) were likewise injected subcutaneously each day, until the vaginal canal opened, with 2 cc. of an extract A "that had been filtered through a porcelain filter." These two extracts were products of Parke, Davis & Company.

In all the series littermate sisters were used as controls. In addition to fifteen untreated controls, 180 others were treated with an amount of normal physiological saline solution corresponding to either the amount of gland transplant or extract injected into the experimental animal. There might be some criticism of controls injected with normal physiological saline solution but the work from this laboratory has shown that the injection of corpus luteum material (Durrant, 1926), theelin, amniotin, whole ovary, and ovarian extract (Durrant et al., unpublished) has failed to produce the characteristic effects shown when pituitary substances were injected.

Immediately after the first injection the rats were put into separate activity cages, similar to those described by Durrant (1924, 1925) and Hitchcock (1925). The cyclometer of each cage was read daily at approximately the same hour.

The animals were weighed weekly throughout the period of experimentation, the first weight being recorded previous to the first injection. There was no appreciable difference in weight between the experimental animals and the controls.

The criteria used in judging sexual maturity were: first, the canalization of the vagina; and second, the bearing of young. Following the injections, the animals were observed hourly to note the time required for the opening of the vaginal canal. After the opening of the canal, vaginal smears were taken daily (Stockard and Papanicolaou, 1927). In this way the presence or absence of a normal oestrual cycle was noted.

In order to investigate the youngest possible age for bearing young, a group of immature female rats were brought to sexual maturity at the age of twenty-three days with gland transplants, in the manner already

described. The smallest males, that were thought to be sexually matured, were introduced into the cages containing the young females. Care was taken to prevent the males from killing or injuring the females, though this happened in some cases. Artificial introduction of sperms was successful with three animals. Two accepted coitus.

In determining the effect of pituitary injections on the size of litters precocious sexually matured females were bred at ages ranging from fifty to sixty days. Pregnant animals were inspected daily and the sexes of the offspring were recorded at birth (Jackson, 1912). The size of litters and the sex ratios obtained were compared with similar data from normal mothers. Data for stillborn young have been included.

TABLE 1

Table showing the average age at which the vaginal canal opened with per cent variation from controls

	TOTAL NUMBER OF ANIMALS	AVERAGE AGE AT OPENING OF VAGINAL CANAL	VARIATION IN EACH SUBDIVISION	AVERAGE AGE AT OPENING OF VAGINAL CANAL IN EACH GROUP	VARIATION IN EACH GROUP
			per cent		*per cent*
Control A	249	34.47		32.86	
Control B	81	28.80			
Group 2 A	220	23.40	67.88	22.79	69.35
Group 2 B	120	21.66	75.21		
Group 3 A	70	21.51	62.58	21.80	66.34
Group 3 B	100	21.00	72.92		
Group 4 A	150	25.31	73.43	24.04	73.16
Group 4 B	70	21.43	74.41		

RESULTS AND DISCUSSION. *Activity.* Since the purpose of this experiment was to determine the effect of the injected material on the voluntary activity of immature rats, the most significant activity would be that recorded before the normal age of puberty. The average age of maturity among the controls was 34.47 days (see table 1). The significant voluntary activity would, therefore, be that of the first two weeks of experimentation. Judged on the activity of these first two weeks the curves obtained when the daily activity of individual experimental animals was plotted failed to show typical oestrous cycles. A few peaks that might be interpreted as heat periods occurred, but since such peaks were more numerous among the controls than among the experimental animals, it must be concluded that the various injected substances failed to produce the cyclic variations in activity characteristic of the matured female.

In judging the effect of the various substances injected on the total voluntary activity records were kept for a period of ten weeks, as it was felt that effects produced before or at puberty might very well persist for some time. The data obtained are shown graphically in charts I, II and III.

It is apparent from chart I that the activity of the animals receiving gland transplants was slightly less than that of the controls. The average for the entire period of the ten weeks is a shade less than ninety per cent of the activity of the control group. This decrease is due largely to a falling off of the activity of the experimental animals in the fifth, sixth and seventh weeks of the experiment.

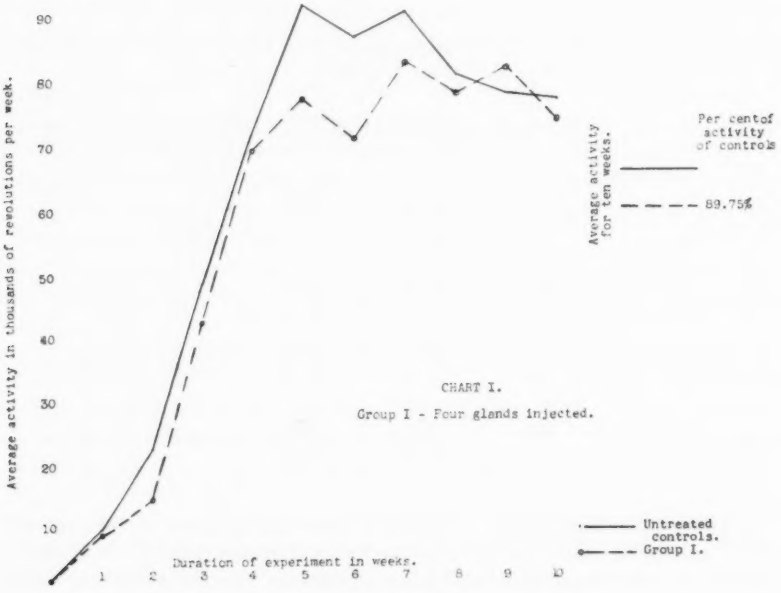
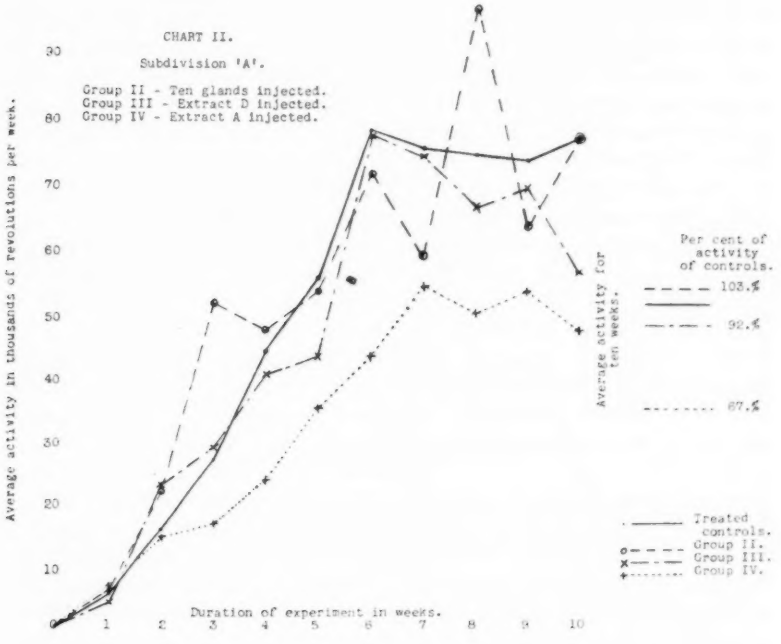


CHART I.

Group I - Four glands injected.

It would seem from an inspection of chart II that the only outstanding effect in voluntary activity produced was a decrease in the activity of the animals receiving extract A. These animals showed an activity only 67 per cent of that of the controls. While there are certain variations in the activity of the animals in groups II and III, it is doubtful if these variations are significant. An inspection of chart III makes the significance of the variations occurring in the activity of groups II and III seem even more doubtful. It must be remembered that these animals were the offspring of female rats that had been prematurely brought to sexual maturity by

gland transplants. On this chart the animals receiving extract D showed an activity greater than the controls, while those receiving gland transplants were slightly less active than the control group. This is just the reverse of the results obtained with the animals whose activity had been plotted in chart II. Therefore, the only conclusion which can be safely drawn is that the injection of pituitary glands or of extract D produces no significant change in the total voluntary activity. However, since the animals injected with extract A showed an activity well below that of the controls

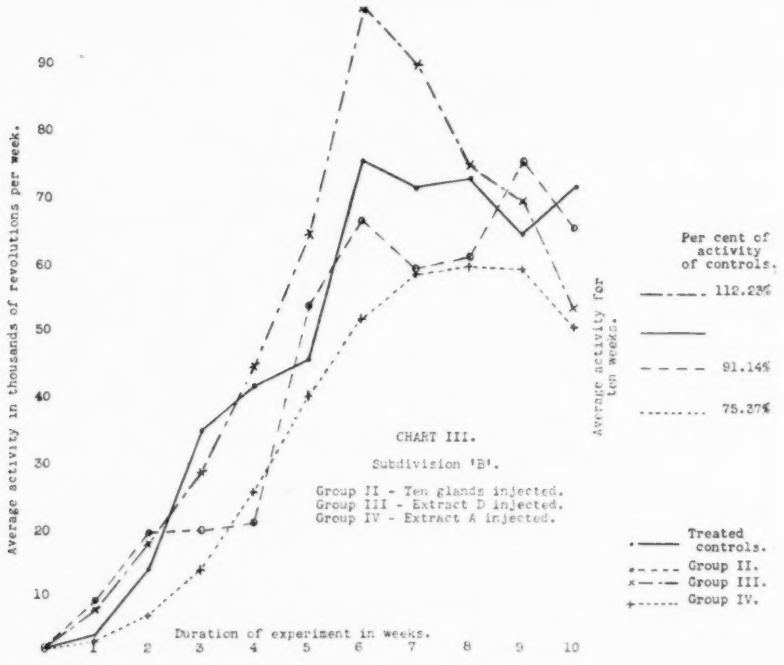


in both groups, it appears justifiable to conclude that this extract depressed the voluntary activity.

Average age at which vaginal canal opened. Table 1 shows the ages at which the vaginal canal opened in the experimental animals and their controls. As in the other parts of the experiment, the animals were divided into two groups. Those animals whose mothers became matured normally constituted subdivision A, while those whose mothers were brought to sexual maturity by the use of pituitary substances constitute subdivision B. In more than 700 animals there was no case in which the treatments used in these experiments failed to induce sexual maturity, as judged by

the canalization of the vagina. Usually two to three days were required. However, with animals twenty days old at the beginning of the treatment, sexual maturity has been induced within seventeen hours by the injection of two gland transplants. No animals less than eighteen days old were used.

It will be noticed that those animals in subdivision B required a shorter treatment than did those in subdivision A. In the case of gland transplants there was an average difference of 1.74 days; with extract A, a



difference of 3.88 days; and with extract D, a difference of 0.54 day. Even the controls showed a decided difference of 5.69 days.

A matured genital system as judged by the canalization of the vagina was secured in thirty animals at nineteen days of age by the injection of 1 cc. of extract D. The animals were treated on their eighteenth day of age. This is considerably younger than any reported case of maturity in normal animals. According to Long and Evans (1922) a normal female rat matures on the average at seventy-two days of age, the range being from thirty-four to one hundred and nine days. According to Donaldson

(1924) the age of puberty is two months. In this experiment the average age at which normal controls matured was 34.47 days.

In this series the opening of the vaginal canal occurred in the experimental animals at ages ranging from nineteen to twenty-eight days. Smith (1927. 1930) reported the youngest age at which he was able to secure a fully matured genital system in the rat to be twenty-two days (the weaning date), treatment having been instituted at the age of fourteen days.

The results obtained from attempts to breed precocious sexually matured animals offer additional evidence that these animals were truly brought to sexual maturity. Five precocious sexually matured females were successfully bred. In three of these the sperm was artificially introduced, while two accepted coitus. These five females bore young at ages ranging from forty-eight to fifty days. Their weights were between 86 and 102 grams. According to Donaldson (1924) the period of gestation is usually from twenty-one to twenty-two days. If this be taken as a standard, the age of impregnation in these females was twenty-six to twenty-eight days. As far as could be found this is the earliest age at which female rats have borne young.

Vaginal smears. Vaginal smears were taken daily on all animals for fifty to seventy consecutive days. In no case was there evidence of a typical cyclic change in the experimental animals. The most frequent smears showed a typical dioestrual cell content. However, frequently a mixed type of cornified and nucleated epithelial cells and leucocytes occurred, in which the proportion of these cells varied from day to day and gave some indications of cyclic changes.

The fact that this mixed type of smear was so frequently found might indicate that instead of the usual period of about five days (Long and Evans, 1922) the oestrous cycle was shortened to a period of one to two days, thus reducing the various stages from days into hours. There is without doubt a decidedly rapid rate of growth of the genital organs in these animals and the accelerated oestrual cycle could be easily explained on this basis.

This accelerated oestrual cycle may explain the failure to obtain activity records indicative of true heat periods. Another possibility is that the luteinization of the ovary produced by the gonad-stimulating hormone of the pituitary interfered with the normal oestrual cycle.

Size of litter. The size of the litters from mothers made sexually mature at an early age was larger in every experimental group than that of the controls. This difference was more pronounced in subdivision B than A (see table 2). Since the animals in subdivision B were the offspring of mothers who had been brought to sexual maturity by gland transplants, the young of these females were the offspring of two generations of treated females.

In subdivision A the average number of young in a litter for the controls was 5.96, based upon forty-five litters. The average number of young born from mothers who had been treated with extract D was 7.34 (21 litters). The average number of young based on twelve litters born from mothers treated with extract A was 10.17, and that from mothers treated with gland transplants was 10.13, taken from twenty-three litters. These differences are significant.

In subdivision B even a greater difference existed. The progeny that received extract D bore on the average 9.10 young per litter (21 litters), those treated with extract A, 9.55, (11 litters), and those treated with gland transplants, 11.50, (20 litters). In twenty-nine control litters the average number of young was 7.97. Combining all the litters in subdivision A we have fifty-six litters with a total of 507 young which is an average of 9.05 young per litter.

TABLE 2

Tabulation of the size of the litters and the sex ratio

	NUMBER OF LITTERS	NUMBER OF MALES	NUMBER OF FEMALES	TOTAL NUMBER OF YOUNG	AVERAGE NUMBER OF YOUNG PER LITTER	NUMBER OF MALES PER 100 FEMALES
Normal mothers:						
Controls	45	164	104	268	5.96	157.68
Experimentals	56	183	324	507	9.05	56.48
Treated mothers:						
Controls	29	123	108	231	7.97	113.89
Experimentals	52	170	356	526	10.11	47.75

Treating the animals in subdivision B in a similar way we have fifty-two litters with a total of 526 young, which is an average of 10.11 animals per litter. It is apparent, therefore, that the effect is more pronounced in the second generation than in the first.

It appears, therefore, that animals treated with pituitary substance are able to transmit some factor that enables their progeny to attain sexual maturity earlier and to show a higher degree of fertility. The transmitted factor is perhaps a greater susceptibility to the hormone of the anterior lobe of the pituitary.

The data contained in table 2 also seem to indicate that injections of pituitary substances decreases the sex ratio. In the three groups of subdivision A there were a total of 183 males to 324 females born in fifty-six litters. This is a ratio of 56.4 males to 100 females. In subdivision B where the young are offspring of two generations of pituitary treatment there were 170 males to 356 females in fifty-two litters. This is a sex

ratio of 47.8 males to 100 females. The sex ratio among the controls of subdivision A was 157.7 males to 100 females, a considerably higher ratio than previously reported. However, in the controls of subdivision B the sex ratio was 113.9 males to 100 females. It must be remembered that while none of the mothers in the control group received treatment, the grandmothers of the control animals in subdivision B had been brought to precocious sexual maturity by gland transplants. A study of these figures seems to indicate that continued treatment in succeeding generations with pituitary substance tends to decrease the relative number of males born. These figures are based on more than 1500 young. While it is perhaps unwise to draw definite conclusions from this number it is felt that the data involved are sufficient to justify the conclusion that treatment with pituitary substance tends to decrease the sex ratio.

SUMMARY

Anterior-pituitary tissue and extracts of the gland were injected into sexually immature female albino rats and the following effects noted:

1. The total voluntary activity was not significantly changed except in the group receiving extract A where there was a marked decrease in the total activity. The voluntary activity of the experimental animals showed no cyclic variations such as those reported by earlier workers on normal sexually matured females.

2. Vaginal smears also failed to show the typical oestrual cycle. The failure to obtain evidence of a typical oestrual cycle may be due to luteinization of the ovary or to a marked shortening of the oestrual cycle.

3. A total of 730 immature female rats received injections of pituitary substance and in every case precocious sexual maturity was produced as evidenced by 1, the canalization of the vagina. This occurred in from seventeen hours to three days following treatment. 2, the experimental animals conceived and bore young. In one case young were produced when the mother was only forty-eight days old.

4. Litters of animals that had received experimental treatment were much larger than the litters of the controls.

5. The sex ratio in the offspring of the experimental animals was much lower than that in the controls, i.e., there was a larger per cent of females in the young of the treated mothers than in the controls.

6. All of the effects noted were more pronounced in the second generation of experimental animals. This may mean that at least a part of the effect is transmitted to the young.

The author wishes to express her appreciation to Dr. L. B. Nice under whose direction the investigation was carried out, and to Parke, Davis & Company for supplying the extracts used.

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STUDIES ON SUBCUTANEOUS ABSORPTION

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In spite of the universal practice of subcutaneous injection in both clinical and experimental work, review of the literature reveals no reference to a study of the rate and mechanism of absorption of subcutaneously injected substances. In an effort to throw light on these questions, determinations have been made as to the amounts of dihydroxyacetone, glucose, fructose, and galactose absorbed in a given time after subcutaneous injection.

METHOD. The method of procedure of these experiments was as follows: known amounts of the four substances, dihydroxyacetone, glucose, fructose, and galactose, were injected subcutaneously in a series of rats. The injections were made into the lumbar region using the Record type of syringe since it could be adjusted so that exactly the same volume, approximately 1 cc., could be injected each time. The same amount of solution was expressed into a flask and analyzed by the method of determination used throughout these experiments. In this way the exact amount of material injected into the rats was determined. The animals were sacrificed at varying periods after injection and the skin was dissected away to recover the unabsorbed material, which was then quantitatively determined. The amount absorbed was then calculated by subtracting the unabsorbed quantity from the total amount injected.

These experiments consist of two series. In the first series, 50 per cent solutions of the four substances were used. The animals were killed immediately and after fifteen, thirty, and forty-five minute periods of absorption. The determinations were performed by the Shaffer-Hartmann method.

The results of the first series of experiments were such that it was deemed essential to perform a second series using more dilute solutions. Both 2.5 per cent and 5.0 per cent solutions of glucose were used. The recovered samples in the second series were analyzed by the method of Hagedorn and Jensen (1923). Control injections of physiological saline were made in

[1] The data here presented are taken from the theses submitted by G. S. Goldman and M. Y. Krosnick in partial fulfillment of the requirements for the degree of Doctor of Medicine, Yale School of Medicine, in 1929 and 1930 respectively.

order to ascertain whether the results were being affected by reducing substances from the tissue fluids, and if so, to what extent. These values were used to correct the apparent values for glucose.

RESULTS. Ninety-two experiments were performed with the concentrated solutions: 40 with glucose, 19 with dihydroxyacetone; 17 with fructose; and 16 with galactose. The results are summarized in table 1. Graphs were constructed for each of the substances plotting the percent absorbed against the period of absorption in minutes, using the data of table 1. In each case the best curve drawn through the four points was a straight line. The final results considered in the discussion are those obtained from the curve rather than the amounts obtained experimentally at the end of forty-five minutes.

The results of the second series of experiments, sixteen with 2.5 per cent glucose and thirty-six with 5.0 per cent solution, are shown in table 2. These values have been corrected for the amounts of reducing substances found after saline injection. The curves to be obtained by plotting these

TABLE 1

Per cent absorbed from 50 per cent solutions in varying periods of time

	15 MINUTES	30 MINUTES	45 MINUTES ACTUAL DETERMINATION	45 MINUTES ESTIMATED FROM CURVE
Dihydroxyacetone	21.6 ±2.2	37.0 ±2.2	57.6 ±1.9	59.3
Glucose	13.7 ±2.0	37.5 ±1.6	45.0 ±1.4	48.0
Galactose	13.4 ±2.8	26.7 ±2.7	37.5 ±2.5	39.2
Fructose	10.9 ±1.8	23.7 ±2.1	43.0 ±2.2	37.0

results are hyperbolic in contrast to the straight lines for concentrated solutions.

DISCUSSION. The absorption of the 50 per cent solutions of triose and the three sugars for a period of 45 minutes is as follows: dihydroxyacetone, 59.3 per cent; glucose, 48.0 per cent; galactose, 39.2 per cent; and fructose; 37.0 per cent. The significance of the differences between these values depends upon the error involved in the injection and recovery of the sugars since the error of the chemical analysis is negligible—less than 0.5 per cent. By referring to table 1, it is found that the average deviations from the mean for each group of experiments range between 1.4 and 2.8. It is obvious, therefore, that the difference between dihydroxyacetone and glucose is significant, as is that between glucose and the other sugars. The difference between galactose and fructose, however, is well within the error of the method.

The first fact indicated by the data of the experiments with 50 per cent solutions is that these substances injected subcutaneously are absorbed

at different rates. Nagano (1902) found that the rate of absorption from the intestine of stereo-isomeric sugars is different, but Hewitt (1924) observed this difference disappeared when the epithelial lining was destroyed with hot saline or fluoride. In this case where no epithelial lining is involved the difference may be ascribed to variations in molecular configurations and activities. The dihydroxyacetone molecule, in particular, is smaller than the hexose molecule. The second point is that the rate of absorption for a given sugar remained constant throughout the duration of the experiment; i.e., the curve of the rate of absorption is a straight line. This phenomenon is regarded by Cori (1925) as characteristic of the absorption of hexoses and pentoses as it occurs in the intestine, lined by a highly specialized epithelial membrane, though Pierce, Osgood, and Polansky (1929) could not confirm such a finding for glucose. Cori and Cori (1929) did not find a constant rate of intestinal absorption of the triose lactic acid. However, no specialized absorptive membrane in the subcutaneous tissues has ever been described; and therefore it seems improbable, in spite of the present evidence, that absorption would take

TABLE 2

Per cent of injected substance absorbed in varying periods of time

	15 MINUTES	30 MINUTES	60 MINUTES
2.5 per cent glucose	40.5 ±1.4	56.9 ±1.8	62.0 ±2.6
5 per cent glucose	23.6 ±0.7	41.1 ±2.6	53.9 ±4.6

place at a constant rate. Instead, the rate of absorption should diminish as the amount of unabsorbed material decreases as in absorption from the peritoneal cavity. It seemed probable that this unexpected result was due to the high concentration of the solutions injected. When the solution is very concentrated as soon as one molecule has been absorbed from the surface of the tissue another takes its place. Thus there may be so many molecules present that there would be no diminution in the rate of absorption for the duration of the experiment. That this is the case is indicated in a few experiments performed with 25 per cent glucose solutions in which the rate of absorption was found to be essentially the same as that for 50 per cent solutions. In view of these results it is probably a fact of lesser importance that the 50 per cent solution of hydroxyacetone used in the experiment contained twice as many molecules as the 50 per cent hexose solutions.

However, when more dilute solutions of glucose were used, namely, 2.5 per cent and 5 per cent, the expected diminishing rate of absorption was found as illustrated by hyperbolic curves obtained by plotting the results. This type of absorption is similar to that observed in the intestine for

lactic acid (Cori and Cori, 1929) and for glucose (Pierce, Osgood, and Polansky, 1929). A diminishing rate of absorption has also been observed in the peritoneal cavity (Cori and Goltz, 1925).

When the animals were killed immediately after the injection of concentrated solutions the total amount of the substance injected was not recovered. The amount not recovered was constant for each substance and so this value could be subtracted from the quantity unrecovered after a definite period of absorption to obtain the amount actually absorbed during that period. The amounts that could not be recovered when the animal was killed immediately are as follows: dihydroxyacetone, 27.0 per cent; glucose, 15.7 per cent; fructose, 11.8 per cent; galactose, 10.7 per cent.

An additional correction factor was required in the experiments with the 5 per cent and 2.5 per cent glucose solutions for quantities of reducing substances, which are significant because of the dilute solutions used, diffuse into the injected solution. It was, therefore, thought advisable to subtract the amount of reducing substances found in control saline injections from the results obtained when glucose was injected. The objection may be made that the infiltration of reducing substances into an injected saline solution is not necessarily indicative of an equal inflow when glucose is injected. However, even without the use of the corrective factor the absorption rate curves for these dilute solutions are hyperbolic, indicating a diminishing rate of absorption, but the use of the corrective factor brings out more pronouncedly the character of the curves.

SUMMARY AND CONCLUSIONS

1. In order to determine the rate of absorption of dihydroxyacetone, glucose, fructose, and galactose, ninety-two experiments were performed in which 50 per cent solutions of these substances were injected subcutaneously. In addition a 2.5 per cent solution of glucose was used in sixteen experiments and a 5 per cent solution in thirty-six.

2. At the end of 45 minutes, the per cent absorbed for each of the 50 per cent solutions was as follows: dihydroxyacetone, 59.3 per cent; glucose, 48.0 per cent; galactose, 39.2 per cent; and fructose, 37.0 per cent. After one hour 62.0 per cent of the 2.5 per cent solution of glucose had been absorbed and 53.9 per cent of the 5 per cent solution.

3. The results indicate first, different rates of absorption for these substances; second, an apparently constant rate of absorption for each substance from a concentrated solution (50 per cent), and third, a diminishing rate of absorption from a dilute solution (5 per cent and 2.5 per cent).

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A STUDY OF CUSHNY'S THEORY OF SULPHATE DIURESIS

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The experiments reported in this paper constitute an attempt to apply Cushny's theory of urine secretion to a specific case. Experiments of this type do not, of course, afford direct evidence of the validity of a theory until they reveal the limits of its applicability, and thus compel modification or rejection. The work was undertaken therefore to see how far the theory could be applied. Cushny's theory was accordingly assumed to be correct and used for making calculations from the experimental data.

As this theory is so well known, it is unnecessary to do more than point out the particular assumptions of the theory made use of in the calculations. Following Rehberg (1926) it is assumed that the creatinine of the urine is derived from the plasma exclusively by filtration and that none of it is reabsorbed. This assumption is used to calculate the amount of filtrate and reabsorbed water. In addition simultaneous samples of blood from the aorta and from the renal vein were taken which permit not only the calculation of the per cent of blood filtered off in the glomeruli, but also estimations of the rate of blood flow through the kidney. We thus have figures for the blood flow, the percentage filtration to the glomeruli as well as the total filtrate, and the amount reabsorbed in the tubules.

Cushny's explanation of sulphate diuresis (p. 149) is that in the first stage the diuresis arises from colloid-dilution and increased flow through the renal capillaries, while as the diuresis passes off the influence of sulphate in the tubules in preventing reabsorption becomes more prominent. It is obvious that now we are in a position to obtain numerical data for these three factors.

METHODS. Experiments were performed on dogs under amytal anesthesia. Although this drug has some action on the kidney (1930) the effects during sulphate diuresis are comparatively small as shown by the latency of water excretion. The amytal was administered intravenously.[1] As soon as complete anesthesia was obtained, the abdomen was opened and the kidneys exposed. In nearly every case one kidney (usually the right)

[1] In some cases sodium amytal was used, for which we are indebted to the Eli Lilly Company.

was isolated by tying off the vessels and the ureters. A cannula was then inserted into the bladder. When both kidneys were allowed to act, ureteral catheters were used. In some cases the carotid blood pressure was recorded. A cannula was then inserted into the femoral vein and through it the animal was given about 200 cc. of 5 per cent sodium sulphate solution and 1.25 gram creatinine dissolved in 25 cc. of water. (The reason for giving the creatinine is the one mentioned by Rehberg; viz., to decrease the error in its estimation in the blood due to the presence of other substances giving the same color.) Collection of urine was begun immediately after the completion of the injection. Samples of blood were taken half an hour later from the abdominal aorta and the renal vein with a needle and syringe, and this was repeated at hourly intervals. The urine was also collected in hourly periods so that the blood samples represent the values in the middle of the periods of urine collection. In half the experiments creatinine estimations were done on whole blood and in the other half plasma was used.

The distribution of creatinine between corpuscles and plasma after creatinine injection has been investigated by Berglund (1922) and by Underhill (1923) who find that the values for plasma and whole blood are usually very nearly the same. We have confirmed this, but we find in agreement with these authors that differences do occur in either direction. Consequently when the figures for whole blood are used, the values for filtrate, etc., may be somewhat in error, but the values for percentage filtered and blood flow will be more exact. When the plasma figures are used, the opposite errors may occur, but it does not seem probable that the magnitude of the error will be such as to make any significant difference in interpretation.

In the first place the percentage of blood filtered may be considered. The exact derivation of the figures for percentage filtration should be borne in mind. They represent the difference in creatinine content of arterial and renal vein blood reckoned as a percentage of the former. The creatinine which is filtered is entirely in the plasma. It is obvious that the process of filtration will not alter the concentration in either plasma or corpuscles, but as the blood travels past the tubules it will be diluted with a considerable volume (perhaps 15 per cent of the total blood volume) of a fluid which is presumably creatinine free. This alters the concentration of the plasma which in turn will produce changes in the corpuscles as a new equilibrium is set up. If this equilibrium is attained by the time the separation of plasma is carried out, then the figures for percentage filtration will be identical whether calculated from whole blood or plasma. The experiments reported at the present time do not settle this point completely, but it will be seen that there is no significant difference between the two sets of values.

It is of interest to compare estimates of per cent filtration derived in this way with those of Mayrs (1922) who derived his figures from the plasma and urine sulphate, the urine volume and blood flow. He finds that the percentage usually lies between 20 and 25 per cent but may vary from 6 to 40 per cent. Gibbs (1928) found that the kidney could remove 30 per cent of the uric acid passing through it. Our figures are somewhat lower. With regard to whole blood the highest value recorded is 20 per cent (expt. 1, table 1). The usual range is between 8 and 15 per cent. In two out of the four experiments the percentage decreases as the diuresis passes off, and in the other two it does not. In experiment 1, for instance, it rises from 12

TABLE 1

Analyses of whole blood

EXPERIMENT NUMBER	WEIGHT OF KIDNEY	SAMPLE NUMBER	URINE VOLUME	URINARY CREATININE	BLOOD CREATININE		FILTRATE	REABSORBED	PER CENT FILTERED	PER CENT NOT REABSORBED	BLOOD FLOW
					Arterial	Renal volume					
	grams		*cc./hr.*	*mgm./hr.*	*mgm./100 cc.*	*mgm./100 cc.*	*cc./hr.*	*cc./hr.*			*cc./gm./hr.*
I	49.5	1	125	281	11.4	10.0	2,465	2,340	12	5.2	406
		2	49	128	9.3	7.4	1,376	1,327	20	3.5	136
		3	39	107	7.4	6.0	1,446	1,407	19	2.4	154
		4	20	43	5.7	4.5	754	734	21	4.4	72
II	69.5	1	137	328	11.8	10.1	2,780	2,643	14	4.9	278
		2	73	146	9.6	8.2	1,521	1,448	14	4.9	150
		3	40	100	8.5	7.9	1,177	1,137	8	3.3	240
		4	27	66	8.0	7.4	825	798	8	3.0	158
III	51	1	35	81	21.8	19.1	372	337	12	9.4	59
		2	1.7	3.6	18.2	16.7	20	18	8	8.5	4.7
		3	1.8	3.3	17.4	16.0	19	17	8	9.5	4.5
IV		1	55	61	13.2	11.6	461	406	14	11.9	3.7*
		2	23	31	11.8	10.0	260	237	16	8.8	1.6*

* Blood flow in litres per hour for the whole kidney.

to 20 as the urine flow decreases from 125 to 49 cc. per hour and then remains stationary while the urine flow drops to less than half this value. The figures for plasma show a maximum of 25 per cent and a minimum of $1\frac{1}{2}$ per cent, the commonest range being between 10 and 15 per cent as in whole blood. The trend is for decreased diuresis to be accompanied by decreased filtration (e.g., expt. 2, table 2), but this is not always the case. In experiment 4 the rate of urine flow decreases from 20 to 6 cc. per hour while the percentage filtered, which changes 14 to 15 per cent, shows no indication of a drop. In experiment 1 the rate of urine formation doubles (22 to 45 cc. per hour) while the percentage filtration is unaltered.

To sum up, it may be said that although in many cases, probably the

majority, the height of diuresis is associated with increased filtration, this is by no means always the case.

The second factor, that of reabsorption in the tubules, can be represented in two ways, of which the more obvious, viz., the percentage reabsorbed, is perhaps not so useful as the percentage not reabsorbed; since variations are much more appreciable with the latter figures. For example, at a constant rate of filtration a decrease in the percentage absorbed from 95 to 90 per cent doubles the amount not reabsorbed, i.e., the urine excreted. Cushny attributes the greater duration of sulphate diuresis to the presence of sulphate in the tubules preventing as complete reabsorption as in its absence.

TABLE 2

Analyses of plasma

EXPERIMENT NUMBER	WEIGHT OF KIDNEY	SAMPLE NUMBER	URINE VOLUME	URINARY CREATININE	PLASMA CREATININE		FILTRATE	REABSORBED	PER CENT FILTERED	PER CENT NOT REABSORBED	BLOOD FLOW
					Arterial	Renal volume					
	grams		*cc./hr.*	*mgm./hr.*	*mgm./100 cc.*	*mgm./100 cc.*	*cc./hr.*	*cc./hr.*			*cc./gm./hr.*
I	53	1	22	47	12.0	9.4	392	370	22	5.6	34
		2	45	72	9.5	7.5	758	713	21	5.9	68
		3	30	57	8.8	7.4	648	618	16	4.6	77
		4	16	40	8.3	7.1	482	466	14	3.3	63
II	37.8	1	64	83	25.7	21.1	323	259	17	19.8	48
		2	15	29	20.8	19.8	139	124	4.8	10.8	77
		3	5.5	5.7	19.2	19.0	29.7	24.2	1.5	18.6	75
III	45	1	98	145	14.0	10.5	1,036	938	25	9.5	92
		2	46	59	10.0	8.5	590	531	15	7.8	87
		3	17	20	9.2	8.0	217	206	13	5.1	38
IV	35	1	71	145	33.3	27.3	436	365	18	16.4	69
		2	9.7	22	25.0	24.0	88	78	4	11.0	63
		3	20	23	21.4	18.5	108	88	14	18.5	23
		4	6	8	20.7	17.6	48	42	15	12.5	7

In three of the experiments with whole blood the percentage not reabsorbed decreases with decreasing urine flow; in experiment 2 this is not the case. The experiments with plasma show much the same picture, but an exception may be pointed out in experiment 2 where the percentage not reabsorbed first falls and then rises with decreasing flow of urine. This phase would doubtless be clearer after a comparison with chloride diuresis (a study it is hoped to make). One can at least say that in nearly all cases increased reabsorption accompanies a decrease in urine flow.

The third factor is the blood flow through the kidney. The highest blood flow recorded is 406 cc. per gram per hour which is less than 7 cc. per gram per minute. This is definitely less than the highest value obtained

by Gibbs (1928) but is of the same order of magnitude as other determinations by him and previous authors. Increased blood flow may produce a larger volume of filtrate in two ways; viz., by an increase in the filtration pressure in each glomerulus; or the increase in both blood flow and that in volume of filtrate may be the expression of a greater number of active glomeruli. It is impossible to differentiate between these in this series of experiments although it is probable that the latter is the more important factor. In table 1 the urine flow and blood flow run together with two exceptions (expts. 1 and 2). In table 2 in rather more than half the samples this is true, but in the others increased urine flow is accompanied by decreased blood flow and vice versa. If one takes the volume of filtrate rather than the volume of urine for comparison, a similar statement may be made.

To sum up, it is impossible to assert that increased urine flow in sulphate diuresis is always accompanied by increased filtration, decreased reabsorption or increased blood flow. One or more of these factors is invariably present, but it is impossible at present to predict which of these is responsible for the greater formation of urine under the conditions of the experiment.

SUMMARY

An attempt is made to apply Cushny's theory to sodium sulphate diuresis. The blood flow and the amounts of fluid filtered in the glomeruli and reabsorbed in the tubules are calculated. Under the conditions of the experiment the diuresis is associated with one or more but not usually all of the following factors: increased blood flow; increased filtration; decreased reabsorption.

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EXPERIMENTAL INTERFERENCE WITH RABBIT EMBRYOS IN THE EARLY STAGES OF THEIR DEVELOPMENT

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The relation of the corpus luteum to the survival of the early mammalian embryo was shown by Corner in 1928 in the first of his series of papers on the *Physiology of the Corpus Luteum.* He showed that if both ovaries of the rabbit are removed 14 to 18 hours after mating, the embryos will nevertheless develop normally to the early blastocyst stage; that normal development will occur and proliferation of the endometrium, when part of both ovaries is removed but one or more corpora lutea remain; and lastly that the removal of the corpora lutea by resection of the ovaries containing them or, in one case, the destruction of one ovary and the corpora lutea in the other by a galvanocautery, leads to the degeneration of the embryos during the first three days of their development. He suggested that, in the last cases cited, early death might be due to a different physiological condition in the tubes resulting from trauma.

Doctor Corner stated, in a personal communication, that it was sometimes impossible to find a trace of the developing eggs 3 to 4 days after cauterizing the copora lutea. A few experiments were carried out at the Biological Laboratory at Cold Spring Harbor, in collaboration with one of us, in an effort to clear up the question of their early disappearance. Later the series was enlarged and a modified method added. Although the results do not have as broad a significance as we first thought that they might have, they are important as being the first record of the deliberate damage by experimental methods to early mammalian embryos.

METHOD AND RESULTS. The animals, common stock rabbits in good condition, were mated twice to one buck and insemination checked by the presence of spermatozoa in the vaginal smear. At periods varying from 15 to 21 hours after this, they were etherized and the ovaries exposed, with aseptic precautions, by flank incisions. With as little manipulation as possible, the corpora lutea were destroyed by a thermocautery. The incisions were closed and the animals were allowed to live for from 41 to 92 hours. They recovered from the operations satisfactorily and appeared in good condition, at the time that they were killed for autopsy by an overdose of ether. The entire reproductive tract was removed and rinsed

in salt solution and then the tubes or the uterine horns were washed out with normal saline according to the method used in the Carnegie Laboratories of Embryology and described by Corner in 1921. The embryos, when found, were compared as to stage of development with those described and illustrated by Gregory (1930). His large series of rabbits were killed at definite periods after mating and the embryos obtained by the above method.

In the 64 rabbits reported by Gregory, he recovered the eggs of 51 in normal condition. He failed in 8 cases to recover all of the ova that had been discharged, as indicated by the presence of recent corpora lutea. In

TABLE 1

Effect of cauterization of the corpora lutea on the young embryos

NUMBER	HOURS AFTER COITUS		CORPORA LUTEA	EMBRYOS	POSITION	CONDITION OF EMBRYOS	NORMAL FOR AGE
	Operation	Autopsy					
1	16	41	6	6	Tubes	3 unsegmented; 3 normal but slightly retarded, 1 6-cell and 2 7-cell	8-cell
13	21	47	8*	8	Tubes	6 normal 16-cell, 2 degenerating	16-cell
2	18	61	12	9	Tubes	Morula stage	Morula
5	18	69	7	4	Tubes	1 morula, 1 9-cell both abnormal, crystals in clear zone; 2 degenerating.	Morula
6	18	91	4	4	Uterus	2 normal blastula, 1 late morula, 1 degenerating	Blastula
3	21	92	8–10	8	Uterus	1 early blastula, 7 late morula. Crystals in clear zone, deformation of membrane	Blastula
4	15	96	4	1	Uterus	Degenerating blastocyst	Blastula

* One not cauterized.

4 animals one or more eggs were abnormal and in one some were retarded in development.

The essential data obtained from our experiments (table 1) are arranged according to the hours after coitus that the animals were killed. Though the series is not large, the fact is rather striking that in not a single instance were all of the discharged ova recovered in normal condition. The eggs of those killed soon after mating suffered slightly less damage. In the earliest, killed in 41 hours, half of the eggs were apparently normal for the period. In the second, killed in 47 hours, 6 of the 8 eggs which had been discharged were normal but the autopsy showed that one of the 8 corpora lutea had not been cauterized. There was one degenerating ovum, how-

ever, found in each tube. In the third, killed in 61 hours, 9 of the 12 ova were recovered in a normal condition but the other 3 were not found. Occasionally there were adhesions and in numbers 1 and 5 there was some hemorrhage about one tube. However, the injuries to the egg occurred when such conditions were not present.

It is possible that a toxic substance produced in the burned tissue was responsible for the damage to the young embryos. The absence of lutein tissue alone could not account for it, since development to the blastula stage will take place after both ovaries are removed, as quoted above.

Seven experiments were performed in order to test the toxicity hypothesis. Rabbits were mated and 19 to 23 hours later the tubes were

TABLE 2

Effect of cauterized tissue, other than the corpus luteum, on the young embryos

NUMBER	HOURS AFTER COITUS		CORPORA LUTEA	EMBRYOS	POSITION	CONDITION OF EMBRYOS	NORMAL FOR AGE
	Operation	Autopsy					
17	19	43.3	5	5	Tube	All 10 to 12 cell, crystals in clear zone of all, 2 embryos showed slight deformation	16-cell
16	21	44.5	7	7	Tube	6 8-cell crystals in clear zone and in embryos, 1 unsegmented	16-cell
14	19	48.5	11	1	Uterus	8-cell degenerating	16-cell in tube
12	20	67	11	2	Uterus	2 morula degenerating	Morula in tube
10	23	70.5	8	0			Morula
11	19	91	4	0			Blastula
9	21	95	7	2	Uterus	1 16-cell degenerating, 1 morula retarded	Blastula

exposed as in the first series (table 2). In order to lessen the manipulation, frequently no effort was made to actually see the ovaries. A piece about the size of a rabbit's ovary was cut from the edge of the incised flank muscle and fastened by a stitch to the fat mass in which the open end of the tube is imbedded. Then this muscle was cauterized, care being taken not to injure the tube, and the incision closed. In no. 17 the muscle was sewed to the inner surface of the flank muscle on the left side, near the tubal end, and then burned, while the right ovary was cauterized in two places. Both ovaries of no. 14 were treated as the right one in 17. In all of these great care was taken not to injure the corpora lutea. A microscopic examination of the ovaries was not made in any of these cases but on gross

inspection at autopsy the corpora lutea all appeared normal. The cauterized muscle was always in place.

It should be noted that, although there was an abundance of lutein tissue present, the ova were not recovered with regularity even in the retarded or degenerating state. In no. 14 one embryo was found in the uterus, 48.5 hours after ovulation. In another instance, no. 12, one was in the uterus in 67 hours. These two instances suggest that the tube was abnormally active, since Gregory gives 80 as the hours after coitus that embryos reach the uterus. In some cases the development was checked at the time of the operation, in others it continued for a few hours. The damage was more serious the longer the rabbit was allowed to live after the operation. These 7 experiments lend weight to the theory that a poisonous substance produced by the burned tissue injures the embryos, which must be very susceptible to chemical abnormalities in the environment.

SUMMARY

The results of 14 experiments are given, in which deliberate injury was done to young rabbit embryos by cauterizing the corpora lutea, or other parts of the ovary or by cauterized muscle fastened near the open end of the tube. The suggestion is made that the burned tissue produces a toxic substance.

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THE STIMULATION OF MUSCLE RESPIRATION BY CARBON MONOXIDE

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It has been shown by Warburg (1927) that carbon monoxide inhibits the respiration of yeast. The completeness of inhibition depends upon the ratio of CO to O and is greater in the dark than in the light (Warburg and Negelein, 1928). Warburg has interpreted the results as indicating that CO combines with the iron-containing respiratory ferment in the same way that it combines with hemoglobin. It has been generally assumed that a similar inhibition of respiration would be found in all cells in CO but very few experiments are on record in which this effect was actually tried.

Warburg himself (1927) measured the aerobic glycolysis in thin sections of rat tissues (liver, chorion, retina, embryo, and Jensen's sarcoma) in light and dark with CO present. He found more aerobic glycolysis in the dark indicating a decreased oxygen consumption due to the CO. As he pointed out, it is difficult in mammalian tissues to get the ratio of CO to O large enough to cause inhibition without decreasing the oxygen supply so much that changes in respiration tend to be obscured. In addition to these observations Keilin (1927) has found inhibition of the indophenol oxidase from yeast and muscle by CO, Reid (1931) has observed inhibition of acetic acid oxidation by bacteria. Tamiya and Tanaka (1930) have reported that the metabolism of acetic acid bacteria is inhibited in the dark but revived in the light, and that the growth of Aspergillus (Tamiya, 1929) and the reduction of methylene blue by bacteria are inhibited by CO, (Tamiya, Hida and Tanaka, 1930). Tamiya (1929) states that the oxygen consumption of Aspergillus Oryzae (per gram) is decreased by CO but Warburg and Kubowitz (1929) found an inhibition. Fujita (1928) has observed also an inhibition of respiration in white blood corpuscles by CO as measured by glycolysis. Schmitt (1930) and Chang and Gerard (1931) have reported inhibition in frog nerve in the dark. The latter authors mention also inhibition in frog muscle mash "with occasional stimulating effects." Haldane (1927) has further observed toxicity of CO to moths which lack hemoglobin and to rats if the tension of CO is raised high enough to

more than saturate the hemoglobin even when the oxygen tension is high enough to ensure adequate oxygen supply in spite of the CO-hemoglobin. Reid (1931) has found inhibition of oxidation of acetic acid by bacteria with CO in the dark but not in the light.

On the other hand CO is without effect on the succinoxidase of muscle (Dixon, 1927) on the oxidation of erythrocytes in the presence of respiratory supplement (Michaelis and Salmon, 1930) on the autoxidation of SH (Dixon, 1928) on the anaerobic oxidation of acetic acid by bacteria in the presence of methylene blue or quinone (Reid, 1931), on peroxidase (Elliott and Sutter, 1932), on glucose oxidase from Aspergillus (Muller, 1929), on the reduction of methylene blue by liver extracts (Tamiya, Hida and Tanaka, 1930). Emerson (1927) has shown that CO does not inhibit the respiration of Chlorella except when the respiration of the plants is increased by immersing them in 1 per cent glucose or other substances. CO is in general non-toxic to invertebrates, microörganisms and plants (Haldane, 1927) and to bacteria (Fischer, Lieske and Winzer, 1931) but is reported toxic to molds (Seeländer). Its behavior is in general like that of nitrogen toward peripheral nerve (Krause, 1930), hens' eggs (Lallemand, 1929), erythrocytes (fragility tests, Mayers, Rivkin and Krasnow, 1930) and tissue cultures (Haggard, 1922).

From this brief review of the literature it would appear that CO does not uniformly inhibit respiration. It should be observed however that a negative result in this respect does not necessarily contradict Warburg's theory for it might merely mean that higher ratios of CO to O should have been used. In yeast Warburg (1926) has shown that the affinity of oxygen for the respiratory ferment is 7 to 14 times as great as that of CO.

Our attention was first directed to this problem by an experiment in which we were endeavoring to learn whether the increased metabolism of frog muscle which is caused by methylene blue would persist unchanged when the respiratory ferment was inhibited by CO. We eventually found that the increase due to methylene blue could be somewhat inhibited by CO but the experiment itself was of much more interest in that it showed a stimulating effect of carbon monoxide on the normal resting metabolism of the frog muscle. We might have been prepared to find no effect of CO in this experiment but there was no precedent for the finding of an actual increase and we were unable to believe the result until it had been repeated many times and controlled in all possible ways. We later obtained evidence that in frog muscle and certain other tissues CO is burned to CO_2 in amount sufficient to account in part at least and probably *in toto* for the stimulation observed.

Method. The oxygen consumption was measured in a differential volumeter (Fenn, 1927) with a capillary holding about 2.5 cu. mm. per cm. and bottles of about 10 cc. capacity. The volumeters were shaken in a

water bath at 22°C. at a rate of about 100 per minute with an excursion of about 3 cm. The bottles were of various sorts but mostly were fitted with two side arms at right angles to one another, one for NaOH and one for reagents which could be tipped on to the tissue as desired. Some were provided with stopcocks for the introduction of gases. When stop cocks were absent the CO mixtures were introduced through the one opening in the bottle and allowed to escape around the ground glass joint which was loosened slightly during the passage of the gas. Great accuracy in the tension of the gas was unnecessary in such cases. CO was prepared in the

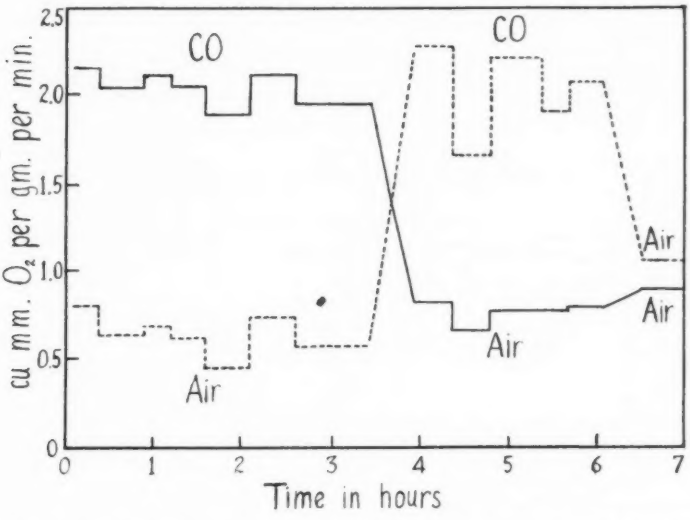

Fig. 1. Rates of gas absorption by two matched frog sartorius muscles in air and in 79 per cent CO, 21 per cent O_2. The muscles were in Ringer's solution in the respirometer in equilibrium with the gases which were changed as indicated. See table 1.

usual way by dropping formic acid on concentrated sulphuric acid or concentrated phosphoric acid. The gas was washed in strong KOH and in some cases through absorbent charcoal in addition in the hope of catching some impurity which might be responsible for the stimulation observed. The mixtures were prepared over water and were analyzed for CO_2 and O_2. When small CO concentrations were used the gas was measured accurately in a burette. The accuracy of the O_2 tensions was therefore carefully checked, the exactness of the CO concentration being of secondary importance.

EXPERIMENTAL RESULTS. The essential fact which we have to report

is illustrated in figure 1. Two sartorius muscles were dissected out from a frog and each muscle was placed in a respirometer in 1 cc. of Ringer's solution. NaOH was added in a side arm to absorb CO_2. One respirometer contained air and the other contained a mixture of 79 per cent CO (approximately) and 21 per cent O. The resulting rates of respiration are plotted in figure 1 and it is apparent that the muscle in CO burns over twice as fast as the control muscle in air. After $3\frac{1}{2}$ hours the gas contents of the two respirometers are interchanged and the muscle which had been in air now burns in CO faster than its fellow in air. Finally both muscles are put into air and the rates of respiration are found to be approximately equal. The effects of CO are not apparently injurious and they are not persistent but quickly reversible.

A summary of seven experiments similar to that in figure 1 is given in table 1. The results show that the average rate of oxygen consumption

TABLE 1

Increased metatolism due to CO in normal frog muscle

IN AIR	IN 79 PER CENT CO, 21 PER CENT O_2	PER CENT INCREASE
cu. mm./gm./min.	*cu. mm./gm./min.*	
0.71*	1.47	107
0.68 (fig. 1)	2.00	206
0.62	2.13	243
0.54	1.44	166
0.74	1.72	132
0.69	1.65	139
0.70	1.67	139
0.67	1.73	162

* Suspended in air. All others were in Ringer's solution.

in air was 0.67 cu. mm. O_2 per gram per minute. Replacing the nitrogen in the air by CO increased the rate to 1.73, an increase of 162 per cent. In making comparisons of this sort measurements in any mixture were continued long enough to reach a constant rate and the final rate attained was used in the tabulation. At least one hour was allowed after introducing a new gas mixture before rates were taken for comparison.

To study this surprising result in more detail a series of experiments was tried, similar in general to the one recorded in figure 1 but using concentrations of O_2 increasing from 0 to 100 per cent the remainder of the mixture being all CO or all nitrogen as the case may be. In each experiment matched sartorius muscles were used from a uniform batch of frogs and the gas mixtures were exchanged after a few hours. The two CO rates and the two control rates were then averaged together and the average percentage increase was thus determined. These averages are plotted

against the tension of oxygen in figure 2. The lower curve represents the control in nitrogen-oxygen mixtures and the upper curve the muscles in carbon monoxide-oxygen mixtures.

The curves coincide of course in 100 per cent oxygen and as the tension of oxygen is diminished there is no change in the oxygen consumption if nitrogen is used as a diluent until the oxygen tension becomes inadequate to supply oxygen by diffusion. The critical tension is in a 10–20 per cent oxygen mixture, thus showing experimentally that in more concentrated mixtures the muscles are well supplied. The sartorius muscles used for these experiments weighed about 70 mgm. When CO is used as a diluent instead of nitrogen the oxygen consumption (apparent) continues to in-

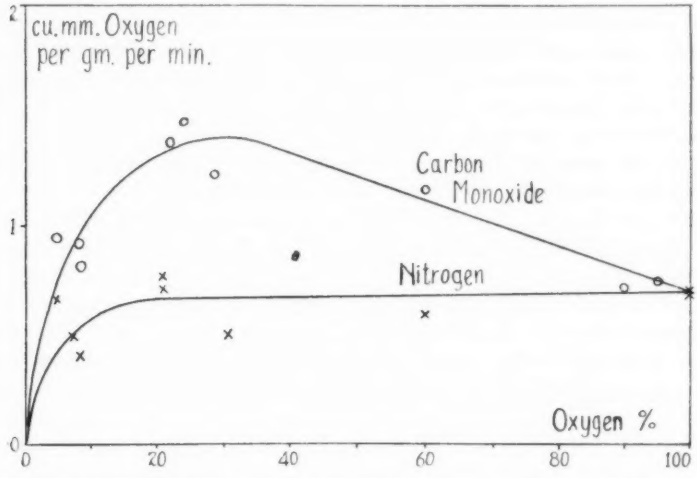

Fig. 2. Gas absorbed by a series of matched sartorius muscles, one in CO and one in N at varying tensions of oxygen.

crease until the oxygen tension becomes a limiting factor, when it must decrease to zero. We have ascertained by independent experiments that the oxygen consumption, or rather the volume diminution does actually go to zero when pure CO is used, i.e., CO is not absorbed in the absence of O_2. We have also tried control experiments in respirometers without tissue and have been able to show that the absorption of CO as formate by the KOH (Warburg, 1931) is not responsible for the greater rates in CO mixtures.

On the suspicion that this CO effect might be due to some error in our experimental procedure we repeated Warburg's experiment with CO and yeast and found a 73 per cent inhibition in the dark in 5 per cent O_2 and 95 per cent CO. Muscle evidently differs from yeast in this respect.

In order to find out whether this increase in the oxygen consumption in CO mixtures was dependent in any way upon the maintenance of normal contractility or irritability of the muscle we tried some experiments with rat muscle strips. The muscle is prepared by dissecting out with the scissors fine strips of the muscle consisting of individual fibres or bundles of fibres which are small enough, according to calculations, to provide sufficient oxygen by diffusion. In order to be sure of this point these muscle strips were kept at 22° during the experiment instead of 37°, their demand for O_2 being thereby diminished. Such muscle strips lose their irritability completely in Ringer's solution in about 15 minutes. In spite of this loss of irritability the metabolism is greater in the presence of CO than in a corresponding N_2-O_2 mixture. Five samples of rat muscle strips were prepared each weighing about 55 mgm. Each sample was placed in a separate respirometer in 1 cc. of Ringer's solution without phosphate buffers. Each respirometer was then filled with a gas mixture containing 20 per cent oxygen and varying amounts of CO up to 80 per cent, the remainder being nitrogen. The rate of oxygen consumption was then followed for over 5 hours. In the absence of CO the rate falls rapidly until it may practically disappear. In the presence of CO the rate is not much higher than in the control at the beginning of the experiment but the rate falls less rapidly and at the end of the first hour of the experiment the effect of the CO is very evident. Graphs of this experiment are shown in figure 3. Each curve represents the oxygen consumption in the different concentrations of CO at a given time, 1, 2, 3, and 5 hours, after the respirometers were put into the water bath. The effect of the CO becomes obvious in concentrations of 20 per cent or greater. One of the chief reasons for the fall in the metabolic rate in the absence of CO is the loss of substrate from the muscles by diffusion. The CO seems to serve to some extent as substitute although even in CO the rate decreases. Another similar experiment was tried with 10 per cent oxygen and Ringer's solution buffered with phosphate. In this case the oxygen consumption in the absence of CO did not fall so low but the increase in metabolism with increase in CO was evident, particularly in concentrations of 20 per cent or more. Lower concentrations were ineffective.

To test further the relation between contractility and the metabolism under CO we have soaked two matched muscles in isotonic KCl for 1 hour until they were quite non-irritable. Each was then put into a separate respirometer, one in approximately 21 per cent O plus 79 per cent CO and the other in air as in the experiment of figure 1. The rate in CO was 1.16 cu. mm. per gram per minute as compared to 0.82 cu. mm. in the control. As a further test the respirometers were removed from the bath after two hours and the gas mixtures were reversed so that the muscle which had been in CO was now put into N and the muscle which had been

in N was now put into CO, both being in 21 per cent O_2 as before. Now the CO muscle showed a rate of 1.66 as compared to 1.45 cu. mm. per gram per minute for the new control. Averaging together the two rates in CO and the two control rates we get figures of 1.41 and 1.13 cu. mm. per gram per minute. The metabolism is therefore increased 25 per cent by the CO. It is evident from this experiment that a completely non-irritable muscle produced by treatment with KCl will still respond to CO with increased metabolism. In one case a pair of spontaneously non-irritable muscles was obtained by omitting the use of Ringer's solution in dissection. In these muscles CO caused a 32 per cent increase in metabolic rate (table 2). The stimulation of metabolism by CO is therefore still present

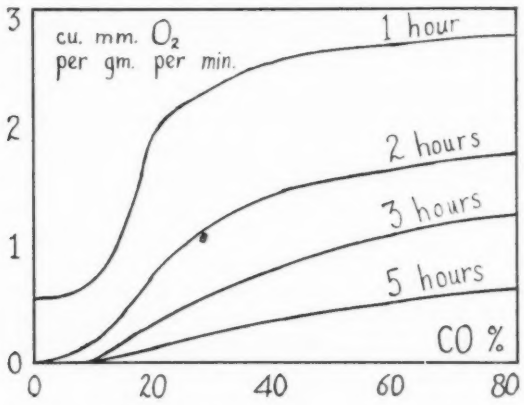

Fig. 3. Oxygen consumption of rat muscle strips in 20 per cent O_2 and increasing tensions of CO at different times (1–5 hrs.) after the start of the experiment. Temp. = 22°C.

in such muscles but it is distinctly less than the 162 per cent increase found in normal muscles.

A considerable number of other experiments of the type just mentioned have been carried out in the investigation of the subject. The results need not be given in detail but it will suffice for the purpose to indicate the percentage increase in metabolism which resulted, it being understood that the complete experiment in every case resembled that described above for KCl. The results are included in table 2.

For purposes of comparison table 2 includes the average figure for normal muscle as found in table 1. In frog muscle mash the increase in metabolism due to CO is only 30 to 60 per cent instead of 50 to 200 per cent as in normal muscles. It does not seem to make any difference whether

boiled muscle juice is added to the brei or not. Such addition tends to maintain the metabolic rate more constant during the experiment. Washing the minced muscle in water destroys its ability to consume oxygen but this ability is revived by the addition of sodium lactate as substrate. In such a preparation CO also serves to increase the metabolic rate 41 per cent. In one well washed muscle mash there was practically no metabolism either with or without CO and the metabolism was not revived by the addition of methylene blue 0.05 per cent or succinic acid 0.07 M.

TABLE 2

Increased metabolism due to CO

	AIR	79 PER CENT CO, 21 PER CENT O_2	PER CENT INCREASE
	cu. mm./gm./min.	*cu. mm./gm./min.*	
1. Whole muscle			
a. Normal average	0.67	1.73	162
b. In KCl 0.9 per cent	1.13	1.41	25
c. Spontaneous non-irritable	1.07	1.41	32
d. In Na-bromacetate 0.03 per cent	0.65	1.41	119
2. Frog muscle mash			
a. In Ringer's	0.93	1.23	32
b. In Ringer's	0.61	0.86	41
c. Add boiled muscle juice	1.01	1.44	43
d. Add boiled muscle juice	0.76	1.25	64
e. Wash once in H_2O. Add Na-lactate	0.27	0.39	41
f. Wash 3 times in H_2O	0–0.07	0–0.05	0
Add methylene blue and succinic acid			
3. Rat muscle			
a. Mash	0.47	0.71	51
b. Strips 37°C.	6.29	7.20	14
c. Strips 22°C.	1.73	2.68	55

Rat muscle strips both at 37°C. and at 22°C. and rat muscle mash also show a stimulation with CO.

The experiments of table 2 show that normal irritability is not necessary for the manifestation of the CO effect. This excess CO metabolism is either a true stimulation of the normal metabolism or a burning of something else. In either case this excess oxidation seems to be mediated by the normal oxidizing system. At any rate loss of materials from the particles of tissue in the muscle mash either by diffusion into the Ringer's solution or by washing with water result in a decrease of the excess CO metabolism as well as of the normal metabolism. Both fractions may be completely abolished by this means but usually the CO metabolism is less

affected so that the percentage increase due to CO becomes progressively larger as the experiment proceeds (fig. 3).

Both the normal and the excess CO metabolism are also decreased more or less equally by suspending the minced muscle in isotonic $CaCl_2$. In one experiment of this type the substitution of $CaCl_2$ for NaCl decreased the metabolic rate in air from 1.48 to 0.09 cu. mm. per gram per minute while the excess rate due to CO was decreased from 0.77 to 0.10. Likewise KCN M/50 decreased both the normal metabolic rate and the excess CO rate of a sartorius muscle to about 10 per cent of the usual value. In K CN solution CO may still cause a considerable percentage increase in metabolism but the absolute increase is much decreased.

Early in the investigation we endeavored to test the possibility that the increased metabolism due to CO was caused by an increased rate of formation of lactic acid. We therefore soaked a muscle in 0.03 per cent sodium bromacetate solution which is sufficient according to the analyses of Hegnauer (appendix to Wright, 1932) on frogs similar to those used by us to abolish completely the formation of lactic acid. On testing the metabolism of such muscles with and without 80 per cent CO we found that the rate of oxygen consumption in the controls was 0.65 cu. mm. per gram per minute, this being increased to 1.41 by the addition of CO (see table 2). An increased lactic acid formation is therefore unnecessary for the CO effect.

As a further test of a possible correlation between the stimulating effect of carbon monoxide and a formation of lactic acid or possibly a breakdown of phospho-creatin we have measured the absorption and elimination of carbon dioxide in oxygen-free mixtures of carbon dioxide with nitrogen and with carbon monoxide. Such experiments in 20 per cent carbon dioxide mixtures have shown always an absorption of carbon dioxide indicating phospho-creatin breakdown, as shown by Meyerhof (1930). There was, however, no detectable difference between the nitrogen and the carbon monoxide mixtures in this respect. In 5 per cent carbon dioxide mixtures there was a preliminary absorption of carbon dioxide followed after two to four hours by a gradual elimination of carbon dioxide indicating the formation of lactic acid. Here again there was no perceptible difference between the muscles in carbon monoxide and those in nitrogen. Some difference may have occurred during the preliminary period of equilibration before it was possible to take readings but this seems improbable.

In some of these experiments oxygen was readmitted to the respirometer after 4 to 6 hours of anaërobiosis and measurements of oxygen consumption were made. These measurements showed that the prolonged stay in carbon monoxide had no lasting after-effects.

To study further this apparent independence of the lactic acid concen-

tration we have tried three experiments in which matched gastrocnemius muscles were put one into pure CO and the other into pure N. Both gases were freed from oxygen by passing them over hot copper. The CO was further washed in NaOH in order to remove the CO_2 formed by the reduction of the copper, and which may be present in considerable amounts. The muscles were suspended over Ringer's solution in glass stoppered tubes with stopcocks at each end. In two cases the muscles were left under such anaërobic conditions for 4 hours at 21–22°C. and were then analyzed for lactic acid by the Friedman, Cotonio and Schafer method. In a third experiment the muscles were at room temperature for 1.5 hours and at 4°C. for 15½ hours before analysis. The lactic acid contents found on analysis in table 3 show a slight increase of lactic acid in the presence of CO. In spite of this result it does not seem possible to explain the stimulating effect of CO by its slight effect on lactic acid formation. We therefore find these experiments on lactic acid formation difficult to interpret.

In one experiment an attempt was made to measure the pH of muscle in carbon monoxide as compared to the control muscles in the hope of explaining in this way the stimulating effect of carbon monoxide on respiration. For this purpose muscles were equilibrated at known tensions of carbon dioxide. The combined carbon dioxide was measured by tipping acid on the muscle and the pH was calculated in the usual way by the Henderson-Hasselbach equation. The muscle in carbon monoxide showed a pH of 6.94 in 3.65 per cent CO_2 and the control muscle showed a pH of 6.89 in 3.46 per cent CO_2.

Further preliminary experiments were tried to discover any possible change in irritability and contractility of muscles which could be attributed to carbon monoxide. For this purpose matched muscles were left, one in air and the other in a mixture of 21 per cent oxygen and 79 per cent carbon monoxide, and the threshold for electrical stimulation was followed. After 5 hours the control muscle showed a 38 per cent increase in irritability whereas the muscle in carbon monoxide showed an increase of 42 per cent. In other experiments the muscles were stimulated rhythmically after periods in carbon monoxide and air and a graphical record of contractions was obtained. In a number of such experiments we could establish no consistent difference between the muscle in carbon monoxide and the control muscle in air. It is possible that further more careful experiments might demonstrate a slight difference in favor of the control muscle. There is no contracture of any sort when a muscle is brought into a CO mixture.

In one experiment the ratio $\frac{Tl}{H}$ was measured for sartorius muscles stimulated by single break shocks by the myothermic methods of A. V.

Hill. T is the tension developed in grams. H is the initial heat in gram centimeters produced by a length of muscle of l cm. In 80 per cent CO and 20 per cent O_2 the ratio was 7.4–7.7 and 7.2 in air which is an insignificant difference. CO has therefore no considerable immediate effect upon the energy required for the development of tension.

The effect of light. The possible effect of light upon the CO metabolism is an important consideration in view of the work of Warburg and Negelein (1928). We have repeatedly measured the stimulating effect of CO upon respiration in 20 per cent O_2 mixtures on comparable muscles, one pair in ordinary daylight and the other pair with black bags tied around the respirometer bottles. Under such conditions we have failed to observe any constant difference attributable to light. Two experiments were tried to test more decisively the effect of light. Two matched muscles were used in each experiment one in CO-O_2 and one in N_2-O_2 mixtures.

TABLE 3

Anaërobic lactic acid formation in CO and in N_2

NUMBER	CO	N_2
	per cent	*per cent*
1	0.062	0.058
2	0.080	0.078
3	0.116	0.088

TABLE 4

Respiratory quotient of frog sartorius muscles

IN AIR	IN 80 PER CENT CO, 20 PER CENT O_2
0.90	0.73
0.86	0.95
0.82	0.72
0.79	0.82
0.92	0.75
0.89	0.80
0.86 Average	0.79 Average

Twenty-one per cent oxygen was used in the first experiment. Measurements of O_2 consumption were made in alternating one-hour periods of light and dark. For dark periods the whole room was darkened and the water bath covered so far as was practicable. For light periods the shades were raised and two 100-Watt electric lights were turned on close to the respirometers. Readings were taken just before a dark period and 5 minutes after the light period began in order that no temperature differences or other similar artifacts could enter in. In each experiment 5 or 6 alternate periods were measured. The rate in the light was consistently slightly higher than the rate in the dark, the ratio of light to dark being on the average 1.08 in CO and 1.2 in air. The absolute rates were always much higher in CO, the increase being 2.64 times in the light and 2.94 times in the dark. It is evident that illumination of this intensity at least is not a factor of importance in comparison to the stimulating effect of CO. The stimulating effect of light in the presence of CO might have

been expected but it is surprising to find more stimulation by light in air than in the CO mixture. In all the experiments reported in this paper the respirometers were in ordinary daylight.

The respiratory quotient in CO. To learn more concerning the nature of the metabolism of muscles in the presence of carbon monoxide we made some measurements of the respiratory quotient. We used for this purpose the methods described by Dickens (1930) in which the carbon dioxide is caught in barium hydrate during the oxygen consumption measurement and then its amount determined by a subsequent acidification. The method need not be described in detail but the results are shown in table 4. Seven measurements of the respiratory quotient of normal sartorius muscles in air gave an average respiratory quotient of 0.869. This figure confirms a similar average obtained previously in a more extensive series (cf. Fenn, 1932). Similar muscles in 21 per cent oxygen and 79 per cent carbon monoxide showed a distinctly lower respiratory quotient of 0.794 average. If one excessively high value of 0.95 is omitted as probably erroneous the average is 0.76. Such a diminution in respiratory quotient could be explained by a burning of carbon monoxide to carbon dioxide. The theoretical R.Q. for this process would be 0.666. A simple calculation shows that a lowering of the R.Q. from 0.86 to 0.76 could be explained by an increase of 111 per cent in the rate of gas absorption, $\frac{1}{3}$ of which would be due to O_2 and $\frac{2}{3}$ due to CO. The extra CO_2 formed would be $\frac{2}{3} \times 111$ or 74 per cent. Thus $\frac{86 + 74}{100 + 111} = \frac{160}{211} = 0.76$. This explanation is reasonable because the average percentage increase in metabolic rate due to CO is as much or more than 111 per cent (table 1). Further confirmatory evidences will be presented in a later paper.

Discussion. It is surprising that this stimulating effect of CO on muscle respiration should not have been discovered earlier. Apparently the effects of CO on muscle have not been previously investigated. In a later paper the effect of CO on other tissues will be described and it will then appear that skeletal and heart muscles are peculiar in this respect. Warburg (1927) tried the effect of CO on a number of rat tissues but he failed to try muscle. Keilin (1927) found that CO inhibited the indophenol oxidase of sheep's heart muscle but he did not try the intact muscle. Hence the experiments herein reported do not constitute a contradiction of any known facts nor in all probability of the familiar theory that CO inhibits respiration. The stimulating effect of CO seems to be in reality a phenomenon which is superimposed upon an inhibition due to CO. Evidence will be presented later to show that the phenomenon is really due to a burning of CO to CO_2. The study of the conditions in the tissues under which this process is able to occur cannot fail to yield valuable information relative to the mechanism of tissue oxidations. The investigation is now being continued with this objective in mind.

SUMMARY

1. Substituting carbon monoxide for the nitrogen of the air surrounding a frog sartorius muscle increases the respiration usually 1.5 to 3 times.

2. A similar though smaller increase in metabolism is produced in the same way in muscle mash of frogs and rats, in frog muscles which are spontaneously non-irritable or non-irritable from isotonic KCl.

3. The increase is diminished but not abolished by loss of the irritability of the muscle; it persists when the formation of lactic acid is inhibited by sodium bromacetate treatment; it cannot be correlated with any changes in irritability or contractility of the muscle.

4. Both the normal respiration and the excess respiration due to CO are diminished to approximately the same extent by KCN, isotonic $CaCl_2$ and by loss of soluble material from the tissue by diffusion or water extraction.

5. Light has no significant effect upon the excess respiration due to CO.

6. The anaerobic acid-base changes of muscle, as followed by the CO_2 absorption or elimination in CO_2 atmospheres were not affected by the presence of CO in place of H or N. CO does not therefore affect significantly the formation of lactic acid or the breakdown of phosphocreatine although a slight increased anaerobic lactic acid content was found in gastrocnemius muscles in CO.

7. The tension developed in single shocks for a given amount of initial heat was not affected by CO.

8. The R.Q. of muscles was lowered from 0.87 to 0.76 by CO. This suggests a burning of CO to CO_2.

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THE BURNING OF CARBON MONOXIDE BY HEART AND SKELETAL MUSCLE

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In a previous paper (1932) we have described experiments indicating that in an intact frog muscle the metabolism is increased between 2 and 3 times when the air surrounding the muscle is replaced by a mixture of 21 per cent O_2 and 79 per cent CO. The purpose of the present paper is to extend these observations to other tissues of both cold and warm-blooded animals and to show that this increased metabolism is not merely an increase in the normal metabolism but rather a CO metabolism in which CO is burned to CO_2 by the consumption of oxygen. The important fact emerges from these experiments that it is only in muscle tissue, either heart or skeletal, that this stimulation of metabolism or the burning of CO can take place to any important extent. The significance of this fact is not at present clear.

SERIES I. THE EFFECT OF CO ON DIFFERENT TISSUES. *Method:* The general technique employed for the oxygen consumption measurements of series 1 has already been described. For the particular experiments herein reported it may be stated that two differential volumeters are used in which are placed two comparable pieces of tissue. In one respirometer is put a mixture of O_2 and CO containing 21 per cent O_2 and, in the other, air. The oxygen tension is therefore the same in both and is adequate to meet the demands of the tissue. Oxygen consumption measurements are then made for 2 to 3 hours after which the gases in the two respirometers are interchanged. The oxygen consumption rates are again measured for a similar period of time. At least 1 hour is allowed before the final rates are obtained which are used in tabulating the results. In muscle there seems to be no after effect due to the period of CO so it seems permissible to average together the two rates so obtained in CO for comparison with the two rates obtained in air. Thus instrumental errors and random differences between the two samples are ruled out. The experiments were all carried on at 22°C. on frog, turtle, and rat tissues. The frogs were *Rana pipiens* or *R. clamitans* and the turtles mostly *Pseudomys elegans.*

Results. By means of the technique described we have made measure-

ments of the gaseous metabolism in a considerable number of different tissues of rats and frogs under the influence of CO. The results are included in table 1. The rates are given in cubic millimeters of gas per gram of fresh tissue per minute. Average figures for thin strips of rat muscle and for intact frog skeletal muscle are included from the previous paper for comparison. It is evident that in skeletal and heart muscle the effect of CO in the light is to cause an increase of 30 to 160 per cent in the metabolism. In other tissues there may be a small increase as in rat tumors, rat liver, frog liver and to some extent nerve, kidney and testis. In frog skin there seems to be a slight diminution even in 80 per cent CO in the light but in 97.6 per cent CO in the dark the inhibition may be as much as 40 per cent. Likewise in nerve in the dark we have one experiment which shows definite inhibition.

Some of these comparisons have been made in ordinary daylight and some in darkness produced by fastening black bags around the experimental bottles. Experiments done in the dark are indicated by a (d) in table 1. In most of these experiments we have not observed any difference which could be safely attributed to the light, and the light and dark experiments have therefore been averaged together. Probably any effect of the light was less than the experimental error.

SERIES II. THE BURNING OF CO. The proof that this increased metabolism is due to the burning of CO depends upon simultaneous measurements of the total diminution in volume, as measured on the differential volumeter, with the oxygen which disappears, as determined by actual gas analysis of the contents of the respirometer before and after a long period of respiration.

Method. The apparatus used for this purpose is shown in figure 1. It is essentially a differential volumeter with rather large bottles holding 32 cc. and fitted with a special stopper for the sampling of the gas and with a special stopcock at the bottom of the bottle for the introduction of mercury. Through this three-way cock mercury is introduced from the leveling bulb when the gas is withdrawn from the respirometer at the end of the experiment. A vertical graduated 1 cc. pipette is also sealed to the third opening on this cock so that small amounts of mercury may be admitted from the pipette at intervals during the experiment. This is done as often as the index drop is drawn to the end of the capillary tube connecting the two bottles of the respirometer. The drop is moved back again in this way every 10 or 20 minutes throughout the day depending upon the amount of tissue in the respirometer bottle. The mercury is thus in direct contact with the Ringer's solution containing the tissues but does not appear to have any injurious effect. The central well in the bottle contains M/1 NaOH for the absorption of CO_2. We have used 1 to 3 grams of tissue in 3 cc. of Ringer's solution in the bottle. This is sufficient to cause a

disappearance of 2 to 4 per cent of the total initial gas contained in the respiration bottle during the course of 5 or 6 hours.

When CO was used about 2 liters of the gas mixture were passed through the respirometer bottle, emerging at the stopper, where the last 100 cc.

TABLE 1

The effect of CO on the gaseous metabolism of different tissues

	CO-O_2	AIR	PER CENT INCREASE	AVERAGE PER CENT INCREASE		CO-O_2	AIR	PER CENT INCREASE	AVERAGE PER CENT INCREASE
	cu.mm. per gm. per min.					*cu.mm. per gm. per min.*			
Rat muscle.....	—	—	—	55	Frog muscle.....	—	—	—	160
Rat heart....(d)	3.68	3.02	22		Frog heart.....	2.33	1.76	33	
Rat heart.....	4.33	3.30	31		Frog heart.....	2.94	1.35	118	
Rat heart.....	6.42	4.81	34		Frog heart.....	2.90	1.12	159	
Rat heart.....	4.58	3.40	35		Frog heart.....	1.96	0.90	113	106
Rat heart.....	2.11	1.36	55	35	Turtle heart...(d)	3.69	1.83	102	
Rat liver.....(d)	4.85	4.70	3		Turtle heart.....	3.02	1.85	63	83
Rat liver.....(d)	5.75	5.05	14		Frog liver†....(d)	2.08	1.72	21	
Rat liver.....(d)	5.70	4.60	24	14	Frog liver.....	2.53	1.96	29	
Rat kidney...(d)	11.29	11.37	−1		Frog liver.....	2.56	2.41	6	
Rat kidney...(d)	10.90	9.80	11		Frog liver.....	2.46	2.26	9	16
Rat kidney.....	10.52	10.30	2		Frog kidney.....	2.79	2.63	6	
Rat kidney.....	11.20	10.00	12		Frog kidney.....	2.69	2.42	11	
Rat kidney.....	9.75	9.71	0		Frog kidney.....	3.23	2.92	11	9
Rat kidney.....	9.91	9.60	3	5	Frog nerve.....	0.84	0.70	20	
Rat spleen.....	3.87	3.72	4		Frog nerve.....	0.84	0.72	16	18
Rat spleen....(d)	3.90	3.55	9	7	Frog nerve....(d)	0.65	0.73	−12**	
Rat spinal cord...	2.57	2.46	4		Frog skin.....	3.45	3.55	−3	
Rat spinal cord...	2.46	2.54	−3	0	Frog skin.....	2.82	3.10	−9	
Rat testis ...(d)	1.77	1.60	10		Frog skin.....	1.77	2.28	−29	−14
Rat testis.....	1.77	1.54	13	12	Frog skin.....(d)	0.99	2.16	−54*	
Rat tumor...(d)	1.12	0.86	30		Frog skin.....(d)	1.08	1.67	−35*	
Rat tumor.....	1.87	1.53	22		Frog stomach.....	0.75	0.77	−3**	
Rat tumor....(d)	2.75	2.53	9		Frog stomach.....	1.83	1.78	+3	
Rat tumor.....	2.75	2.44	13	19	Frog stomach.....	1.81	1.93	−6	−2
					Yeast.....(d)	0.3	1.16	−74	−74

The CO-O_2 mixture consisted of approximately 21 per cent O_2 and 79 per cent CO except where otherwise noted.

(d) = done in darkness.

* 2.4 per cent O_2.

** Approximately 10 per cent O_2 in both.

† The first 2 experiments with frog liver were done with large pieces and the last two with thin slices. The greater injury may cause less CO burning.

were caught over mercury in a sampling tube for analysis. The final oxygen content was determined at the end of the experiment, usually by withdrawing the gas directly into the analysis burette of a Henderson-Haldane gas analyzer. The analyzer was checked by analyzing room air before each experiment except in some of the earlier experiments. Two analyses were made of the gas in the respirometer at the beginning of the experiment and two at the end. In most of the experiments a second sample was taken for analysis at the end of the observation period after the first sample had been analyzed.

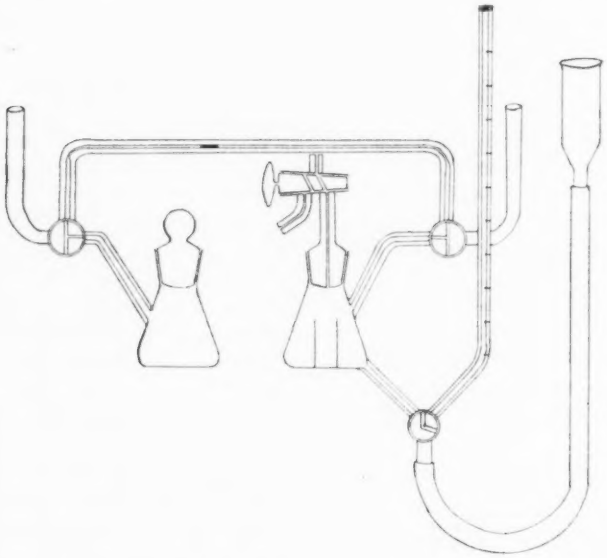

Fig. 1. Apparatus used for simultaneous measurement of total gas absorbed by volume and oxygen consumed by gas analysis.

The respiration period was calculated to the time of first sampling beginning at the time the CO mixture stopped passing. Since the respirometer was placed in the bath before the CO mixture was introduced equilibrium could be established rapidly after stopping the flow of gas and volume measurements could be begun within 2 to 4 minutes. The total volume diminution is measured directly on the vertical 1 cc. pipette with the addition of two short extrapolations at the beginning and end of the experimental period and a correction to take account of the position of the drop on the capillary tube.

The calculation of the amount of oxygen consumed during the experiment is made by the following equation

$$a\ V - x = b(V - x) \text{ whence } x = \frac{(a - b)\ V}{1 - b}$$

where V is the volume of the experimental bottle, a and b are the percentage compositions of the gas in oxygen at the beginning and the end respectively of the experimental period, and x is the volume of gas consumed (saturated with water vapor in equilibrium with the Ringer's solution in the respirometer at the temperature of the bath and the barometric pressure which obtained when the experiment was begun). In measuring V we included the narrow tubing leading from the experimental bottle up to the stopcock in the stopper and up to the bend in the index capillary. The total volume diminution as measured on the vertical 1 cc. pipette also refers to the moist gas as it exists in the respirometer bottle. The results have been so expressed in the tables without further correction to standard conditions.

Results. The observations of series II made by the method just described are collected in table 2. The 4th and 5th columns show the oxygen consumed as measured by gas analysis, calculations being made from the results of the analysis of the first and second samples respectively. The 6th column gives the total gas absorbed during the same period of time as measured by the amount of mercury which had to be introduced into the apparatus to keep the index drop in position. This total gas usually exceeds the oxygen consumed (4th column) by a certain amount which is shown in the 7th column expressed in percent of the oxygen. This represents the amount of CO which is consumed. Column 3 gives the average rate of oxygen consumption per gram per minute during the experimental period as calculated from the data of columns 1, 2 and 4.

The first 5 experiments of table 2 are controls showing the results obtained with frog muscle and turtle heart in air. The total gas absorbed agrees with the amount of oxygen consumed within 4 per cent. In the last 6 experiments using frog skin and liver and rat spleen and liver the total gaseous metabolism can be accounted for by the oxygen consumed within plus or minus 10 per cent. These are the tissues in which, according to table 1, there is little or no stimulation of the respiration by CO. In all the other experiments in table 2 the oxygen consumed fails to account for the total gas absorbed by amounts which are well outside the experimental error and range from 9 to 101 per cent. Thus in skeletal muscle and heart muscle there is some other gas absorbed besides oxygen. Since CO is the only other gas present it must be concluded that CO is absorbed. Since it has been shown in a previous paper that CO is not absorbed when O_2 is absent it must be concluded that some of the O_2 consumed is used for the

burning of the CO. The fact that this consumption of CO is found only in those tissues in which CO stimulates respiration, and then in amounts which are comparable in magnitude to the increase in metabolism observed, is strong evidence that all of the stimulation of respiration by CO is caused by the burning of CO to CO_2.

TABLE 2

Difference between total gas consumed and oxygen consumed, or the amount of CO burned

TISSUES	1	2	3	4	5	6	7
	WEIGHT OF TISSUE	TIME	O_2 PER GM. PER MIN.	OXYGEN CONSUMED		TOTAL GAS USED	DIFFERENCE
				1st	2nd		
Controls in air							
	gms.	*min.*	*cu. mm.*	*cc.*	*cc.*	*cc.*	*per cent*
Frog muscles, intact	0.95	389	0.84	0.31	0.36	0.32	3.2
Frog muscles, cut up	3.55	411	0.76	1.10	1.14	1.14	2.7
Frog muscles, cut up	3.37	390	0.96	1.37	(1.36)	1.42	3.6
Frog muscles, cut up	4.13	393	0.72	1.16	1.23	1.20	3.4
Turtle heart strips	1.92	423	0.79	0.72	0.77	0.72	0
Experiments in 79 per cent CO + 21 per cent O_2							
Frog muscle, intact	2.32	393	0.65	0.59		1.09	85.0
Frog muscle, intact	2.39	361	0.66	0.55		1.04	89.0
Frog muscle, intact	2.29	369	0.92	0.78		1.08	38.0
Frog muscle, intact	2.15	347	0.94	0.70		0.91	30.0
Frog muscle, cut up	3.20	394	0.70	0.88		1.11	26.0
Frog muscle, cut up	2.93	370	0.93	1.01	1.09	1.14	13.0
Frog muscle, cut up	3.73	256	0.69	0.66	0.71	0.78	18.0
Frog muscle, cut up	2.49	350	1.02	0.89	0.90	1.01	14.0
Frog muscle, cut up	3.48	371	0.83	1.07	1.09	1.30	22.0
Frog muscle, cut up	3.31	338	0.72	0.81	0.85	0.89	10.0
Frog muscle, cut up	2.51	320	1.02	0.82	1.00	0.98	20.0
Rat heart slices	1.08	296	2.72	0.87	0.92	0.98	13.0
Rat heart slices	1.24	301	3.65	1.36	1.45	1.67	23.0
Turtle heart strips	2.22	362	0.66	0.53	0.58	1.06	100.0
Turtle heart strips	1.17	395	0.76	0.35	0.45	0.64	83.0
Frog skin	3.63	299	0.80	0.87	0.92	0.81	−7.0
Frog skin	2.92	318	0.93	0.86	0.92	0.88	2.3
Frog liver	2.00	306	2.04	1.25	1.27	1.27	1.6
Frog liver	1.51	334	2.20	1.11	1.29	1.13	1.8
Rat liver	2.45	320	1.54	1.21	1.25	1.33	9.9
Rat spleen	1.04	287	3.65	1.09	1.15	0.99	−9.2

Most of the muscles used in the experiments of table 2 were not intact muscles but were simply pieces about the size of a sartorius muscle cut from the thigh of the frog. It was not realized at that time that the condition of the muscle would make any particular difference and it is difficult to get

enough tissue by any other means. These experiments on "cut up" muscles gave an average increase in metabolism due to CO combustion of only 17 per cent on the average. This was so much less than the 162 per cent found in intact muscles in the experiments of series I that we tried more experiments with intact muscles. This required the dissection of the sartorius, ileofibularis, and semitendinosus muscles from 4 frogs making 24 small muscles in all, each weighing 70 to 100 mgm. When these were used immediately after dissection the average rates of oxygen consumption were 0.92 and 0.94 and the percentage increases were 37 and 30 per cent respectively. Both of these rates are a little high for normal muscles. In a third experiment the 24 muscles were allowed to soak in 500 cc. of continuously aerated Ringer's solution in the cold room over night and were put into the respirometer in the morning. These muscles had a lower rate of oxygen consumption of 0.63 and a higher CO increase of 89 per cent and must be regarded as the most nearly normal of all those used in table 2. Another similar experiment showed an increase of 85%.

When 24 small muscles are put into 2 cc. of Ringer's solution immediately after dissection their rate of oxygen consumption never falls to normal levels. This is perhaps due tc the K liberated from cells injured in dissection. Under such conditions CO does not seem to burn well.

Before leaving the discussion of table 2 attention should be drawn to column 5 which contains the figures obtained for the O_2 consumed from the analysis of the second sample withdrawn from the respirometer about 20 minutes after the first sample. During this interval the respirometer was not in the water bath and was not shaken so that it is impossible to say exactly how much more oxygen should have been consumed in this time. Furthermore after removing one sample for analysis the volume of gas remaining is much diminished so that further metabolism causes a greater percentage change in oxygen content than previously. For these reasons the second sample was used merely to confirm in an approximate way the results of the first sample. Calculation showed that the difference between the first and second samples was less than it should have been if respiration had continued at its previous rate. Probably the absence of shaking diminished the interchange between the solution and the gas.

In one of the control experiments (indicated in brackets, table 2) there is some obvious error for the oxygen consumed as calculated from the second sample (column 5) is less than that calculated from the first sample (column 4). It is possible that the error was in the analysis of the first sample rather than the second in which case the oxygen consumed would check even better with the total gas absorbed. In other cases in table 2 the difference between the oxygen consumed as calculated from the two samples is impossibly large. We have assumed, however, that the first sample is more likely to be correct and have used the first figure for com-

parisons. In the experiments on intact frog muscles only one sample was taken; this one sample was large enough for duplicate analyses. The figures obtained by this method are correspondingly more accurate. It is regrettable that the same procedure was not followed in all the experiments.

By way of summary a comparison is made in table 3 of the experiments series I and series II. In series I the total gas absorption only is measured on two samples, one in CO-O and the other in N-O mixtures, the differences giving the percentage increase. In the second series the oxygen consumed as measured by gas analysis is compared with the total gas absorbed the difference, expressed in percent, being CO consumed. The parallelism, while by no means exact, is good enough to be quite impressive. Discrepancies are due to variations in the condition of the tissue and to changes in the environment which determine whether CO can be burned or not.

TABLE 3

Comparison showing that the amount of CO consumed is sufficient to account for the stimulation of metabolism caused by CO

	INCREASE IN GAS CONSUMED IN CO— SERIES I	CO CONSUMED IN PER CENT OF O_2— SERIES II
	per cent	*per cent*
Skeletel muscle, intact	162	90
Skeletel muscle, cut up (av.)	44	17
Cold blooded heart	98	92
Rat heart	35	18
Frog skin	−14	−2
Frog liver	16	+2
Rat liver	14	+10
Rat spleen	7	−9

That this is true for muscle has already been indicated. In liver it has appeared that large relatively uninjured pieces burn CO better than thin slices (table 1). Further work is necessary, however, before conclusions can be drawn for tissues other than muscles. In considering table 3 it should be remembered that the percentage increase in total gas absorption should be one-third larger than the CO consumed because one extra mol of oxygen must be absorbed to burn each mol of CO. From table 3 it may be concluded that the increased gas exchange in CO mixtures is due to the burning of CO.

Discussion. One of the earliest theories of carbon monoxide poisoning suggested that CO acts in the body as it acts toward metals, i.e., as a reducing agent taking oxygen away from the blood and becoming itself oxidized and liberating CO_2 and heat in toxic quantities (Chénot, 1854).

In spite of the obvious error in this theory it now appears that this oxidation of CO can in fact occur in the mammalian body but it is doubtful whether this process is of any significance in actual cases of CO poisoning where the concentration of CO is so small in comparison with that used in these experiments. The oxidation of CO to CO_2 is also well known among the bacteria some of which (Carboxydomonas) derive their energy from this reaction. More recently it has been shown by Negelein (1931) that CO can be burned to CO_2 by hemin in $\frac{m}{1}$ NaOH if the O tension is low enough to leave the Fe only partially oxidized. Presumably therefore Warburg's iron-containing respiratory ferment is responsible for the oxidation of CO in tissues. This is confirmed by some observations previously recorded showing that both the normal metabolism and the CO metabolism are similarly affected by KCN, $CaCl_2$, and water extraction. It is possible that the increase in metabolism in *Chlorella* by CO as described by Emerson (1927) may also be due to a burning of CO by these plant cells.

SUMMARY

1. The effect of CO upon the gaseous metabolism of a variety of tissues has been studied in rats and frogs. Marked increases of 160 and 98 per cent are found in skeletal muscle and heart muscle respectively. Other tissues show smaller and less constant increases (liver, Jensen rat sarcoma, spleen, nerve, testis, cord, blood) or inhibition (frog skin).

2. By means of simultaneous determinations of total gas absorbed and of oxygen consumed (by gas analysis) it is shown that the increased gas absorption observed in CO is due to a burning of CO to CO_2 which likewise occurs to an appreciable extent only in heart and skeletal muscle.

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THE POSTERIOR PITUITARY HORMONE IN METABOLISM

III. The Effect of Pitressin and Pituitrin upon the Lipoid Distribution

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Coope (1925) and Chamberlain (1925) observed marked changes in the contents of liver fatty acids in rabbits after pituitrin administration. Himwich and co-workers (1930a, 1930b) found decreases in the plasma fat of dogs dosed with pituitrin, pitocin, or pitressin. That a more complete picture might be presented, fat determinations were included in the carbohydrate studies made by the authors on rabbits dosed with pitressin or pituitrin (Bischoff and Long, 1931). In this series there were three groups of experiments—the first group of seven medullary adrenalectomized rabbits had been studied for varying lengths of time. The second group was deglycogenated by a dose of synthalin previous to the experiment. The third group had been standardized for insulin dosage. The doses of pituitrin were not as large nor the interval before death as long as the dosage and time interval in Coope and Chamberlain's experiments. Tissue analyses were confined to the liver and muscle. While such analyses in themselves might give valuable information as to the mobilization and utilization of fat, a knowledge of what happened to the sum total of fat in the body was necessary before definite conclusions might be drawn. Since the rabbit does not lend itself readily to such determinations, the study was continued upon mice.

Experimental. *Rabbit.* The moisture and fat content was determined in the liver and muscle of rabbits under the varying conditions described above. The sacrolumbalis muscle was excised from a freshly killed rabbit, freed from surrounding fat and connective tissue, cut into narrow strips and dried in an oven at 85° to 90°C. until a constant weight was attained. The dried muscle was then ground and samples weighed for ether extraction by the Soxhlet method. The whole liver minus the gall bladder and obvious connective tissue was dried and samples taken for Soxhlet extraction. Substances other than fats are obtained by ether extraction but the results obtained for the same tissues should be comparable. In a few experiments blood for fatty acid and cholesterol determina-

tions was taken before and after the substance being studied was administered. The fatty acids were determined by Bloor's method (1928) and since they include cholesterol, the latter was determined also by the method of Bloor, Pelkan and Allen (1922) and subtracted from the first value.

TABLE 1

The effect of pitressin and pituitrin upon the moisture and ether soluble content of muscle and liver of rabbits under various experimental conditions

RABBIT NUMBER	WEIGHT OF RABBIT	CONDITION OF ANIMAL	DOSAGE PER KILOGRAM (SUBCUTANEOUSLY)	HOURS BEFORE KILLING	MUSCLE		LIVER		BLOOD (MGM. PER 100 CC.)			
									Cholesterol		Fatty acids	
					Moisture	Fat	Moisture	Fat	Before dosage	After dosage	Before dosage	After dosage
Group I												
					per cent	*per cent*	*per cent*	*per cent*				
3	1.93	Adrenalectomized	None		73.7	0.60	66.7	5.0		49		268
4	1.87	Adrenalectomized	None		79.6	0.30	69.4	4.6				
5	3.18	Adrenalectomized	None		75.1	0.23	71.2	3.6				
9	2.27	Adrenalectomized	15 units pituitrin	6	74.8	0.51	74.4	3.9	52	63	331	338
10	2.5	Adrenalectomized	15 units pitressin	6	78.1	0.58	73.1	4.2	77	56	243	195
1	2.16	Adrenalectomized	15 units pitressin	5	81.7	0.33	72.2	5.1	40	33	298	303
2	2.55	Adrenalectomized	15 units pitressin	5	77.7	0.36	71.3	4.6	46	46	176	185
Group II												
2	2.09	Deglycogenated by synthelin	3 gm. glucose at convulsion time	6	75.0	0.86	81.2	4.4				Plus cholesterol 190
3	2.1	Deglycogenated by synthelin	3 gm. glucose. 15 u. pitressin	6	75.0	0.87	85.7	3.2				
1	2.16	Deglycogenated by synthelin	3 gm. glucose. 15 u. pitressin. 1 mgm. adrenalin	6	74.2	0.82	77.8	4.6				Plus cholesterol 232
Group III												
14	1.81	Standardized for insulin	4 units insulin	1½	76.1	0.42	69.3	4.7		53		460
16	1.7	Standardized for insulin	3 units insulin	1½	74.9	0.47	68.8	5.4		68		435
15	2.0	Standardized for insulin	4 u. insulin. 15 u. pitressin	1½	74.1	0.52	69.5	5.9		47		456

Of the seven adrenalectomized rabbits, three were used as controls, one received 15 units of pituitrin, and three 15 units of pitressin per kilo subcutaneously 5 to 6 hours before sacrifice. Since the rabbits weighed around 2 to 3 kilos, the dosage of pitressin and pituitrin was under 2 cc. Coope and Chamberlain (1925) gave doses of 3 to 4 cc. of pituitrin but do not state the size of their rabbits. It will be noted from table 1 that the

ether soluble fraction of the liver and muscle varied as much or more for the controls as for the rabbits receiving pitressin and the one receiving pituitrin. The moisture content did not show any significant variation with the possible exception of the liver which showed an increased moisture content after pitressin.

In the second group of three rabbits, which had been given 6 mgm. of synthalin, 3 grams of glucose per kilo were administered when convulsions intervened, rabbit 2 acting as control, rabbit 3 receiving 15 units of pitressin per kilo, and rabbit 1, 15 units of pitressin per kilo, and 1 mgm. of adrenalin. The rabbits were sacrificed 5 hours later. It will be noted that the muscle fat content of the synthalin group ranges somewhat higher than that found for the other group. The ether soluble fats of the liver are within the average range.

In the third group, rabbits 14, 15 and 16 had received insulin over a period of several weeks until the smallest doses causing a convulsion were determined. On the day of the experiment, rabbit 14 received a subconvulsive dose, rabbits 16 and 15 a convulsive dose, and in addition rabbit 15 received 15 units of pitressin which prevented a convulsion. All of the rabbits were killed at the time required for rabbit 16 to convulse, which was approximately $1\frac{1}{2}$ hours. The ether soluble liver and muscle fraction as well as the water content were within the normal variation.

The cholesterol and fatty acid values of the blood determined before and after dosage did not vary significantly in any of the groups of experiments.

Mice. Fat was determined on whole mice by a modification of Mottram's method. Male mice approximately 6 weeks of age with an average weight of 15 grams were used. Four controls and four dosed animals were run simultaneously so that mice of the same age and litter might be compared.

The mice were killed by a blow on the head, weighed and put into 20 cc. of 60 per cent KOH, heated in a boiling water bath until all except the bones were dissolved. Twenty cubic centimeters of 90 per cent alcohol were added and the heating continued for an hour. The contents of the flasks were washed into 300 cc. Kjeldahl flasks with hot water and cooled. Thirty-five cubic centimeters of 40 per cent H_2SO_4 were cautiously added, keeping the flask cooled under running water. After heating a few minutes in a water bath the flasks were again cooled in ice water. Fifty cubic centimeters of petroleum ether were added with a pipette and the flasks tightly stopped with tin-foil-covered corks moistened with glycerin. The flasks were then shaken some 30 times at intervals of a minute each time for about 10 seconds, filled with water so that the petroleum ether layer came up into the neck of the flask, then allowed to stand for an hour or more, giving the neck of the flask a sharp blow with the hand occasionally to facilitate the emulsion to separate. Usually a 40 cc. aliquot can be pipetted off into tared flasks and the ether evaporated. While the flask is still full of fumes, it is put into a vacuum desiccator, the remainder of the ether vapor being removed in this way. The weight became constant on the second or third day.

The results are tabulated in groups (table 2), the dosed mice with the controls, which were killed at the same time, and represented litter mates or mice of the same age. There was much variation between different groups of controls, so that each group of dosed mice must be compared with the controls killed at the same time. The first group of dosed mice received 0.25 unit of Parke-Davis' pitressin at 6 and 3 hours before killing, having fasted 24 hours in all. The variation among these mice was as great as their controls, and the means showed no significant difference. Another group received four doses of 0.25 unit every 3 hours during the last half

TABLE 2

Lipoid substances according to Mottram's method of whole mice after various doses of pitressin and of controls

	0.5 PRESSOR UNIT OF PITRESSIN*	CONTROL	1.0 PRESSOR UNIT OF PITRESSIN†	CONTROL	24 PRESSOR UNITS OF PITRESSIN†	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Group 1	3.3	2.8	2.1	3.2	11.6	
	3.0	2.8	2.1	4.1	6.0	
	4.1	2.9	2.1	4.0	10.8	
	4.1	3.1	2.1	3.0	11.6	
Group 2	3.0		6.5	4.8	7.0	
	3.3	2.8	6.6	4.1	5.1	5.3
	3.4	2.5	2.1	3.4	5.4	
			5.3	2.9		
Group 3	3.3		3.1		2.5	
	2.5	3.5	4.5		2.2	2.8
	4.0	3.1	1.8		2.1	
	5.0	4.0	2.8			
	3.5±0.21	3.1±0.15	3.4±0.44	3.7±0.21	6.4±1.6	

* Starved 24 hours, dosed 6 hours before killng.

† Starved 24 hours, dosed 12 hours before killing.

of their 24-hour fast, 1.0 pressor unit giving no more definite results. The variation was greater than for those receiving 0.5 unit. In fact, the results were paradoxical, some values falling below the lowest control range while others exceeded the highest values of the control range. The means of the dosed and control groups showed no significant difference.

To determine whether the dose given was pharmaceutical, blood sugars were determined for mice after giving them doses of pitressin. Since it was impossible to obtain enough blood from the tail to determine even micro sugars, the animal was sacrificed by cutting the throat. The blood was quickly collected with a micro pipette and put into the acid tungstate

solution. The value obtained by this procedure was compared with the value obtained for a composite sample taken from the tails of two or more mice. Values of 104 and 96 mgm. per 100 cc. were obtained on the composite sample as compared to 102 mgm. when collected from the throat. Fifteen minutes after a second dose of 0.25 unit of pitressin given 3 hours apart, the blood sugar was 190. Fifteen minutes after 1.0 unit had been given in four doses of 0.25 unit each every 3 hours, the value was 160 mgm., 30 minutes later 210 mgm., and after 45 minutes, 133 mgm.

The lethal dose of pitressin for the mouse was found to be around 30 pressor units, given in three divided doses 4 hours apart. If pitressin affects fat metabolism, the results should be exaggerated with an excessive dose. Accordingly, a sublethal dose of 24 pressor units was given in three doses of 8 units each over a period of 12 hours, after a 12-hour fasting period. At the end of the 24 hours, the mice were killed and subjected to analysis by the modified Mottram method. The first group showed very high results but unfortunately no control was run. The next group was high but the control was just as high, and the next group gave as low figures as had been obtained in other groups. The petroleum ether soluble content of the saponified mouse is apparently subject to a marked individual variation and values obtained without a control group are of little value. The mean for the lipoid substances of the whole group dosed with 24 units was 6.4 $\pm$1.6 per cent, which is higher than the mean for groups of lesser dosage. Only two controls were done for this group with values of 5.3 per cent and 2.8 per cent, giving a slightly higher average than the other groups.

Discussion. Since pituitrin has found extensive clinical use in the treatment of obesity, evidence from animal experimentation that fat metabolism is affected by pituitrin has been sought. It must be admitted that convincing evidence is as yet entirely lacking. The work of Coope and Chamberlain (1925), which demonstrated an increase in liver fat, and the experiments of Raab (1926) and of Himwich and his co-workers (1930a, 1930b) on blood fat decreases, indicate changes in fat mobilization rather than fat utilization. The decrease in blood fat observed in the initial stages of pituitrin activity is at least in part explained by the hydration of the blood, a well-established pituitrin effect. Coope and Chamberlain are not inclined to regard the increases in liver fat observed by them as due to "fatty infiltration," although massive doses of pituitrin were used. The doses used in the present studies while less are nevertheless pharmaceutical as shown by the hyperglycemia, urination and diarrhea which followed each injection. Rony and Ching (1931) were unable to find any significant effect of pituitrin upon an alimentary lipemia. Our data show that in the rabbit pharmaceutical doses of pitressin did not affect the hepatic or peripheral fat deposits significantly. If there is mobilization or a change

in utilization, the fat content of the liver and muscles is held within certain limits.

Using Rubner's figures, the amount of fat oxidized by an 18 gm. mouse in a 4-hour period, assuming that fat was the only substance metabolized, would be 0.068 gram. On the basis of the fat analysis obtained in the present studies, the standard deviation of the mean of the fat content of a group of eight mice fasted 24 hours is ± 0.04 gram. The difference in the means of two series determined was twice this deviation. It is therefore apparent that profound changes in fat metabolism (100 per cent change) might actually take place, without detection, because of the factor of individual variation. The method (analysis of total fat content) is therefore eliminated as an attack to the problem of establishing the effect of the hormones (insulin, adrenalin, etc., as well as pituitrin) upon fat utilization over the active period of a single intravenous or subcutaneous dose.

In the present studies the active period was extended to 12 hours by repetition of dosage. On the basis of an eighteen gram mouse, the joint standard deviation of the mean (square root of the sum of the squares) for the control and dosed series equals ± 0.087 gram fat. Using the same assumption for the 12-hour period that was used for the 4-hour period, the maximum amount of fat that could be oxidized by the controls is 0.204 grams. A change in fat content corresponding to a 42 per cent change in metabolism would therefore be without significance.

SUMMARY

The moisture content, the ether soluble fats of the muscle and liver of rabbits determined under various conditions after the administration of pitressin, pituitrin and insulin did not vary significantly. The fatty acids and cholesterol values of the blood determined upon the same animals before and after dosage came within normal variations.

No significant changes in the total lipoid content of mice were noted following pitressin administration over a wide range of dosage. The individual variation of the total lipoid content was found to be of a magnitude so great that a 100 per cent change in fat metabolism over a 4-hour period would not affect the fat content significantly.

Doses of 0.5 unit and 1.0 unit of pitressin cause a hyperglycemia in mice when given in divided doses of 0.25 unit 3 hours apart.

The lethal dose of pitressin for mice was found to be around 30 pressor units when given in divided doses over a 12-hour period.

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THE FORMATION OF GLYCOGEN FOLLOWING PANCREATECTOMY

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The present study was undertaken to determine if the depancreatized organism has the ability to form glycogen in muscle and liver. Most, if not all, of the investigations that have been reported on the formation of glycogen have been done in the presence of complete diabetes of recently depancreatized or eviscerated animals. Objection is raised to this approach to the problem in that such animals are not necessarily in the diabetic state because of the short time since removal of the pancreas or because they are not in good physiologic condition. With this in mind, it was deemed advisable to allow the animals to recover completely from removal of the pancreas before attempting to induce the formation of glycogen in the liver and skeletal muscles. The conclusion that this process requires insulin is puzzling, even if much of the present experimental evidence is in favor of this view, since on discontinuing the administration of insulin to the depancreatized dog, the glycogen content of the skeletal muscles is not appreciably diminished.

In spite of the strides which have been made in the last decade on problems concerning carbohydrate metabolism, we must admit that even now we do not know the fundamental difference between the diabetic and the nondiabetic states. It is possible that this difference is quantitative rather than qualitative. It seemed possible to us that the animal with diabetes of maximal degree might retain the ability to form glycogen, provided proper conditions were established.

The conditions which seemed essential to our investigators were: 1, pancreas completely removed; 2, maintenance of the animal under insulin treatment until it was in good condition and the operative wound had healed, and 3, withdrawal of insulin for a period of five days before beginning the final part of the experiment. Although it cannot be stated that some insulin was not present at the end of five days after its withdrawal from the depancreatized animal, it is not practical to wait much longer because of the severity of the diabetes. The recent investigations of Best, Jephcott and Scott would indicate that animals under the conditions of those in our experiments would not have a significant amount of insulin.

PREVIOUS INVESTIGATIONS. Considerable data have been recorded in support of the belief that the organism with complete diabetes is incapable of forming glycogen, although a few investigators have published experiments contradictory to this view. Soskin (14) concluded that the dog with complete diabetes has little if any impairment in ability to oxidize carbohydrate. Chaikoff (4) determined the average glycogen content of the skeletal muscles in fasted, depancreatized dogs on the third and fifth days after withdrawal of insulin. He noted that the difference in the two determinations was not sufficient to account for the energy which had been expended during the time between the two estimations, and concluded that in order to supply this energy, glycogen must have been formed in the muscle, unless it can be assumed that other substances than glycogen can serve as the fuel for the production of energy in muscle.

Fisher and Lackey studied the glycogen content of the heart muscle, skeletal muscle and liver in normal dogs and in diabetic dogs on the same standard diet. The diabetic dogs did not receive insulin. It was found that the glycogen in the heart muscles of diabetic dogs was considerably higher than in normal dogs, and the converse was true of the skeletal muscles and liver. Cruickshank (6) also demonstrated an increase in the glycogen content of heart muscle following pancreatectomy.

Macleod and Markowitz (12) found that five days after the last administration of food or insulin to a depancreatized dog the skeletal muscles contained large amounts of glycogen. Macleod (10) (11) has shown that, although glycogen is always present in the skeletal muscles of depancreatized dogs, the injection of insulin effects a deposition of more glycogen in such muscles.

Choi, working on cats, found that glycogen does not form in muscle after evisceration regardless of the concentration of blood sugar. Best, Hoet and Marks (1) found that in the recently depancreatized cat the intravenous infusion of glucose is not followed by an increase in glycogen in muscles unless insulin is injected.

Markowitz, Mann and Bollman (13), working on recently depancreatized nephrectomized dogs, found it difficult to demonstrate an increase of glycogen in muscles subsequent to the injection of large quantities of glucose, although in some cases glycogen was undoubtedly formed. The objection to this, and to other similar work, is that insulin may have been stored in the tissues in sufficient quantities to effect the formation of glycogen. Fraser and Macleod endeavored to find some form of carbohydrate which would induce the formation of glycogen in muscle in the eviscerated preparation, but they were unsuccessful except for one experiment in which dihydroxyacetone was injected. Kermack, Lambie and Slater (9) also were able to demonstrate an increase of glycogen in muscles in recently eviscerated cats when this form of carbohydrate was injected. They also

observed an increase of glycogen in muscles following the infusion of glucose.

The most recent work on the subject is by Bodo, Tui and Farber (3). They studied the changes in glycogen in the liver following the injection of glucose in animals that had been depancreatized forty-eight hours previously. They found slight but definite increase of glycogen.

METHODS AND MATERIAL. The experiments were all performed on dogs. Operative procedures, including the taking of specimens of muscle were carried out under ether anesthesia. The pancreas was removed according to a method employed in our laboratories. The essentials of the method are: 1, to remove the organ by blunt dissection with an aneurysm needle; 2, to take care never to break the gland so that the danger of leaving bits of pancreatic tissue is reduced, and 3, never to ligate blood vessels. After removing the pancreas the duodenum was covered with omentum, and the abdomen was closed in the usual manner. After operation the dogs received intravenous injections of glucose twice daily for three days following pancreatectomy, but nothing by mouth during this interval. Subsequently they received a liquid diet of milk and syrup, and finally, on the fifth day a solid diet of ground meat and crackers, to which a little raw pancreas was added. Injections of insulin were begun on the day following operation.

After the abdominal wound had healed entirely, which usually required several weeks, food and insulin were withheld for five days before the actual experiment was begun. Glucose-nitrogen ratios were estimated to determine whether the animals were in a complete state of diabetes at the end of the fasting period, before beginning the actual experiment. Determinations of nitrogen were made according to the method described by Kjeldahl, and estimations of sugar in the urine were done by the method described by Benedict.

In all experiments control specimens were taken either from the same muscle or from the corresponding muscle of the opposite side of the body. That such a specimen serves as an adequate control has been shown in a previous paper. Determinations of the glycogen content were done by a modified Pflüger method. The injections of glucose were given by the intravenous route. After the animals had been killed careful search was made for remaining bits of pancreatic tissue throughout the abdominal cavity, special attention being paid to the walls of the intestine and gall bladder, as well as to the bed of the pancreas. Pancreatic remnants were not found in the experiments reported. The livers of all of the animals were characteristic of the liver of the depancreatized animal.

RESULTS. The detailed results of each experiment are given in the protocols. In summary they are as follows: fifteen experiments were performed on depancreatized dogs in which the glycogen of the liver and muscles was

determined before and after the intravenous infusion of glucose. In nine experiments glycogen formed in the liver in significant quantities, it was slightly increased in one experiment, and was diminished in five experiments. The glycogen in muscles was decreased in eight experiments; it was slightly increased in one experiment, and was significantly increased in five experiments.

EXPERIMANTAL DATA. *Experiment 1.* The weight of the dog was 7.5 kgm. The abdominal wound was completely healed one month following pancreatectomy. The first specimens of muscle and liver were secured at 9:02 to 9:25 a.m. From 10:00 until 11:58 a.m. 0.00075 gram of epinephrine for each kilogram of body weight per hour was injected intravenously continuously. The second specimens of muscle and liver were secured as 12:00 m. to 12:15 p.m. From 12:30 p.m. until 4:30 p.m. the dog received 2 grams of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 2.2:1. The entire body of the animal shivered when the final specimens were taken. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	9:16 a.m.	0.093
Liver	12:10 p.m.	0.075
Liver	4:42 p.m.	0.068
Left sartorius	9:13 a.m.	0.222
Right sartorius	12:03 p.m.	0.080
Right sartorius	4:35 p.m.	0.032
Left quadriceps	9:14 a.m.	0.153
Right quadriceps	12:05 p.m.	0.090
Right quadriceps	4:31 p.m.	0.021
Left quadriceps	4:36 p.m.	0.024
Left gracilis	9:15 a.m.	0.085
Right gracilis	12:06 p.m.	0.071
Left gracilis	4:32 p.m.	0.032
Right gracilis	4:38 p.m.	0.026
Left adductor	9:17 a.m.	0.194
Right adductor	12:07 p.m.	0.124
Left adductor	4:34 p.m.	0.008
Right adductor	4:40 p.m.	0.100

Experiment 2. The weight of the dog was 7.5 kgm. The wound was entirely healed six weeks following pancreatectomy. From 9:00 to 11:00 a.m. 0.1 mgm. of epinephrine was injected intravenously for each kilogram of body weight per hour, and from 2:00 to 5:00 p.m. glucose was injected

intravenously at the rate of 2.0 grams for each kilogram of body weight per hour. The first specimens of muscle and liver were secured at 1:30 to 1:58 p.m. The second specimens were obtained after the animal had been killed. The glucose-nitrogen ratio during the twelve hour period prior to beginning the experiment was 2.8:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	1:48 p.m.	0.245
Liver	5:15 a.m.	0.206
Right sartorius	1:40 p.m.	0.018
Left sartorius	5:01 a.m.	0.002
Right quadriceps	1:41 p.m.	0.092
Left quadriceps	5:04 a.m.	0.001
Right gracilis	1:43 p.m.	0.003
Left gracilis	5:22 a.m.	0.001
Right adductor	1:44 p.m.	0.122
Left adductor	5:10 a.m.	0.002

Experiment 3. The weight of the dog was 8.5 kgm. The abdominal wound had healed six weeks subsequent to pancreatectomy. Epinephrine was not injected, but the animal was placed in the cold to promote glycogenolysis. The first specimens of muscle were secured at 2:20 to 2:45 p.m. The final specimens were removed after the animal had been killed. Glucose was not administered on the first day of the experiment, but from 9:00 a.m. on the second day until 5:30 a.m. on the third day the animal received 1.5 grams of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio prior to beginning the experiment was 2.7:1. Estimations of glycogen were as follows:

Muscle	*Time*	*Date*	*Glycogen, per cent*
Right sartorius	2:30 p.m.	December 10	0.025
Left sartorius	6:05 a.m.	December 12	0.043
Right gracilis	2:32 p.m.	December 10	0.027
Left gracilis	6:06 a.m.	December 12	0.039
Right adductor	2:35 p.m.	December 10	0.065
Left adductor	6:09 a.m.	December 12	0.072
Right quadriceps	2:33 p.m.	December 10	0.067
Left quadriceps	6:09 a.m.	December 12	0.075

Experiment 4. The weight of the dog was 8.9 kgm. The abdominal wound had healed three weeks following pancreatectomy. The animal

was placed in the cold for a period of eight hours, after which the first specimens of muscle were removed under local anesthesia. Subsequently glucose was injected intravenously at the rate of 1.5 grams for each kilogram of body weight per hour for twelve hours. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 2.1:1. The second specimens were secured at the time of death. Estimations of glycogen were as follows:

Muscle	*Time*	*Glycogen, per cent*
Left sartorius	7:02 p.m.	0.090
Right sartorius	6:35 a.m.	0.040
Left quadriceps	7:04 p.m.	0.080
Right quadriceps	6:37 a.m.	0.060
Left gracilis	7:05 p.m.	0.076
Right gracilis	6:38 a.m.	0.010
Left adductor	7:06 p.m.	0.116
Right adductor	6:39 a.m.	0.070

Experiment 5. The weight of the dog was 4.4 kgm. The abdominal wound had healed in a little less than three weeks. The first specimens of muscle and liver were secured at 8:40 to 9:10 a.m. From 9:00 a.m. to 3:00 p.m., 1.0 gram of glucose for each kilogram of body weight per hour was injected intravenously. The second specimens were then removed. The glucose-nitrogen ratio prior to beginning the experiment was 1.2:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:58 a.m.	0.204
Liver	3:30 p.m.	0.140
Right sartorius	9:06 a.m.	0.030
Right sartorius	3:35 p.m.	0.020
Right quadriceps	9:08 a.m.	0.030
Right quadriceps	3:37 p.m.	0.060
Right gracilis	9:09 a.m.	0.020
Right gracilis	3:38 p.m.	0.010
Right adductor	9:10 a.m.	0.104
Right adductor	3:39 p.m.	0.070

Experiment 6. The weight of the dog was 11.4 kgm. The wound had completely healed at the end of three weeks. Specimens of muscle and liver were secured at 8:20 to 9:00 a.m. From 9:00 a.m. until 3:00 p.m.,

1.5 grams of glucose for each kilogram of body weight was injected intravenously. The animal remained in good condition throughout the experiment except that there was considerable shivering of the extremities. The glucose-nitrogen ratio was 3.0:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:50 a.m.	0.140
Liver	3:00 p.m.	1.070
Right sartorius	8:55 a.m.	0.255
Right sartorius	3:03 p.m.	0.019
Right quadriceps	8:56 a.m.	0.185
Right quadriceps	3:04 p.m.	0.086
Right gracilis	8:57 a.m.	0.455
Right gracilis	3:06 p.m.	0.013
Right adductor	8:58 a.m.	0.668
Right adductor	3:06 p.m.	0.250

Experiment 7. The weight of the dog was 9.4 kgm. The wound healed slowly, and it was not possible to begin the experiment until seven weeks after pancreatectomy. The first specimens of muscle and liver were secured at 8:30 to 9:05 a.m. The final specimens were obtained after the animal had been killed. From 9:30 a.m. to 4:30 p.m. the animal received 2 grams of glucose intravenously for each kilogram of body weight. The glucose-nitrogen ratio during the eighteen hours previous to the injections was 2.56:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:42 a.m.	0.086
Liver	4:30 p.m.	0.290
Right sartorius	8:53 a.m.	0.072
Left sartorius	4:32 p.m.	0.221
Right gracilis	8:57 a.m.	0.151
Left gracilis	4:34 p.m.	0.238
Right adductor	8:58 a.m.	0.050
Left adductor	4:36 p.m.	0.326
Right quadriceps	8:55 a.m.	0.151
Left quadriceps	4.33 p.m.	0.218

Experiment 8. The weight of the dog was 13.4 kgm. Nine weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of liver and muscle were secured at 8:30 to 9:00 a.m. The

final specimens were obtained after the animal had been killed. From 9:00 a.m. to 9:00 p.m. 0.5 gram of glucose for each kilogram of body weight per hour was administered intravenously. The animal remained in good condition throughout the experiment except for marked shivering of the entire body. The glucose-nitrogen ratio during the twelve hours preceding the injection was 2.6:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:45 a.m.	0.528
Liver	9:10 p.m.	0.994
Left quadriceps	8:50 a.m.	0.024
Right quadriceps	9:12 p.m.	0.032
Left adductor	8:52 a.m.	0.028
Right adductor	9:14 p.m.	0.052

Experiment 9. The weight of the dog was 5.6 kgm. Three weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of muscle and liver were secured at 8:35 to 8:57 a.m. From 9:30 a.m. to 3:00 p.m., 1.0 gram of glucose for each kilogram of body weight was injected intravenously. The glucose-nitrogen ratio during the twenty-four hours previous to beginning the experiment was 3.4:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:42 a.m.	1.00
Liver	8:42 a.m.	0.98
Liver	3:00 p.m.	1.26
Liver	3:00 p.m.	1.28
Right quadriceps	8:57 a.m.	0.16
Right quadriceps	8:57 a.m.	0.16
Left quadriceps	3:02 p.m.	0.06
Left quadriceps	3:02 p.m.	0.06
Right adductor	8:58 a.m.	0.20
Right adductor	8:58 a.m.	0.19
Left adductor	3:04 p.m.	0.07
Left adductor	3:04 p.m.	0.05

Experiment 10. The weight of the dog was 7.0 kgm. Three weeks after pancreatectomy the abdominal wound had entirely healed. The first specimens of muscle and liver were secured at 8:30 to 9:00 a.m. The second specimens were obtained after the animal had been killed. From 9:00 a.m. to 7:00 p.m., 0.5 gram of glucose for each kilogram of body weight per hour was injected intravenously. The glucose-nitrogen ratio during the twelve hours preceding the experiment was 2.6:1. The animal

shivered excessively during the hour previous to obtaining the final specimens. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	8:42 a.m.	0.04
Liver	8:42 a.m.	0.05
Liver	7:00 p.m.	0.12
Liver	7:00 p.m.	0.15
Left adductor	8:52 a.m.	0.42
Left adductor	8:52 a.m.	0.53
Right adductor	7:12 p.m.	0.08
Right adductor	7:12 p.m.	0.09
Left quadriceps	8:52 a.m.	0.22
Left quadriceps	8:52 a.m.	0.20
Right quadriceps	8:08 p.m.	0.02

Experiment 11. The weight of the dog was 5.0 kgm. The wound had completely healed three weeks following pancreatectomy. The first specimens of muscle and liver were secured at 12:30 to 1:00 p.m. The final specimens were obtained after the animal had been killed. From 1:00 p.m. to 1:00 a.m. the animal received 0.5 gram of glucose intravenously for each kilogram of body weight per hour. Unlike previous experiments, the dog used in the present study was kept under artificial heat during the course of the experiment to avoid the shivering which had been noted repeatedly in other dogs. The glucose-nitrogen ratio prior to beginning the experiment was 2.9:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	12:40 p.m.	0.123
Liver	12:40 p.m.	0.131
Liver	1:05 a.m.	0.563
Liver	1:05 a.m.	0.555
Left adductor	12:46 p.m.	0.090
Left adductor	12:46 p.m.	0.086
Right adductor	1:09 a.m.	0.358
Right adductor	1:09 a.m.	0.376
Left quadriceps	12:50 p.m.	0.075
Left quadriceps	12:50 p.m.	0.068
Right quadriceps	1:13 a.m.	0.323
Right quadriceps	1:13 a.m.	0.342

Experiment 12. The weight of the dog was 7.3 kgm. Three weeks after pancreatectomy the wound had completely healed. The first specimens of liver and muscle were secured at 1:35 to 2:10 p.m. The final specimens were obtained after the animal had been killed. During the experiment the

animal was kept warm and there was no gross shivering. From 2:30 p.m. until 2:00 a.m. the dog received 0.5 gram of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio was 3.2:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	1:43 p.m.	0.136
Liver	1:43 p.m.	0.121
Liver	2:10 a.m.	0.736
Liver	2:10 a.m.	0.718
Left quadriceps	1:58 p.m.	0.137
Left quadriceps	1:58 p.m.	0.145
Right quadriceps	2:17 a.m.	0.421
Right quadriceps	2:17 a.m.	0.398
Left adductor	2:03 p.m.	0.167
Left adductor	2:03 p.m.	0.156
Right adductor	2:21 a.m.	0.532
Right adductor	2:21 a.m.	0.518

Experiment 13. The weight of the dog was 7.3 kgm. Two weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of liver were secured at 1:30 to 2:00 p.m. The final specimens were obtained after the animal had been killed. Glucose was injected intravenously at the rate of 0.5 gram for each kilogram of body weight from 8:00 p.m. until 4:00 a.m. During the experiment the animal was kept warm. The glucose-nitrogen ratio prior to beginning the experiment was 3.2:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	7:23 p.m.	0.086
Liver	7:23 p.m.	0.078
Liver	4:05 a.m.	0.834
Liver	4:05 a.m.	0.819
Left adductor	7:42 p.m.	0.101
Left adductor	7:42 p.m.	0.093
Right adductor	4:11 a.m.	0.509
Right adductor	4:11 a.m.	0.516
Left quadriceps	7:46 p.m.	0.081
Left quadriceps	7:46 p.m.	0.091
Right quadriceps	4:14 a.m.	0.438
Right quadriceps	4:14 a.m.	0.453

Experiment 14. The weight of the dog was 8.8 kgm. Twelve days after pancreatectomy the abdominal wound had almost healed. The first specimens of muscle and liver were secured at 7:20 to 7:50 p.m. From 8:00 p.m. to 3:30 a.m. 0.5 gram of glucose was injected intravenously for

each kilogram of body weight per hour. The animal was kept warm throughout the experiment. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 3.1:1. The animal shivered despite the fact that it was kept warm. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	7:15 p.m.	0.201
Liver	7:15 p.m.	0.196
Liver	3.35 a.m.	0.181
Liver	3.35 a.m.	0.169
Right adductor	7:31 p.m.	0.087
Right adductor	7:31 p.m.	0.077
Left adductor	3:42 p.m.	0.023
Left adductor	3:42 p.m.	0.017
Right quadriceps	7:35 p.m.	0.074
Right quadriceps	7:35 p.m.	0.083
Left quadriceps	3:45 a.m.	0.042
Left quadriceps	3:45 a.m.	0.038

Experiment 15. The weight of the dog was 5.5 kgm. The wound had completely healed four weeks after pancreatectomy. The first specimens of muscle and liver were secured at 6:20 to 6:55 p.m. The second specimens were secured after glucose had been injected intravenously for a period of six hours at the rate of 0.5 gram for each kilogram of body weight per hour. After the second specimens had been obtained, glucose was injected intravenously at the rate of 1.0 gram for each kilogram of body weight per hour, and in addition insulin was injected intravenously at the rate of 5 clinical units per hour. After six hours of such treatment the animal was killed and the final specimens were obtained. The glucose-nitrogen ratio during the twelve hours previous to beginning the experiment was 3.0:1. Estimations of glycogen were as follows:

Muscle or liver	*Time*	*Glycogen, per cent*
Liver	6:29 p.m.	0.096
Liver	6:29 p.m.	0.087
Liver	12:15 a.m.	0.431
Liver	12:15 a.m.	0.426
Liver	6:10 a.m.	1.236
Liver	6:10 a.m.	1.189
Right quadriceps	6:43 p.m.	0.019
Right quadriceps	6:43 p.m.	0.030
Left quadriceps	12:24 a.m.	0.327
Left quadriceps	12:24 a.m.	0.311
Right quadriceps	6:17 a.m.	0.934
Right quadriceps	6:17 a.m.	0.958

Muscle or liver	*Time*	*Glycogen, per cent*
Right adductor	6:47 p.m.	0.231
Right adductor	6:47 p.m.	0.241
Left adductor	12:28 a.m.	0.398
Left adductor	12:28 a.m.	0.415
Right adductor	6:20 a.m.	1.126
Right adductor	6:20 a.m.	1.200

COMMENT. It may be noted that the results of the foregoing experiments are not uniform. In the first experiments two factors were not adequately controlled: 1, too large doses of glucose were administered which produced an unfavorable general reaction in the animal, and 2, in the earlier experiments shivering was not controlled. By keeping the dogs warm shivering was either obliterated or minimized.

It was found that during administration of glucose, glycogen in the liver decreased in five animals; it was slightly, although not significantly increased in one animal, and it was appreciably augmented in nine animals. The glycogen in muscles was decreased in eight animals; it was slightly, but not significantly increased in one animal, and it was definitely increased in five animals.

These results are interesting because the positive data are much more convincing than the negative. In none of the reported experiments was pancreatic tissue found after careful search at necropsy, and the animals appeared to be in a complete state of diabetes.

It appears from these experiments that the depancreatized organism is capable of forming glycogen in both liver and muscle, but it is not formed in amounts comparable with that of the normal animal or of the diabetic animal under treatment with insulin. The question of oxidation of this stored carbohydrate is beyond the scope of this paper.

SUMMARY

The depancreatized dog, five days after withdrawal of insulin, is capable of forming glycogen in both skeletal muscle and liver, although at a rate far below that of a normal dog.

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THE ANTIRACHITIC EFFICIENCY OF NEW ORLEANS SUNSHINE[1]

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The chief factor in the seasonal incidence of rickets is the seasonal variation of the sun's altitude. This determines the quality and quantity of ultra violet, particularly that shorter than 313 mμ. The actual number of sunshine hours is of relatively little significance, as illustrated, for instance, by the conditions in the Panama Canal Zone, where rickets is practically non-existent, but where the yearly sunshine is less than in New York City, and less evenly distributed (Hess, 1925).

New Orleans, a sub-tropical city, is of particular interest in this connection. Although the mean temperature of New Orleans is high, the Weather Bureau records show an average for the last forty years of only 58 per cent sunshine out of the total possible number of hours, an amount not greater than that in many other cities in this country. Furthermore, being practically at sea level and having a very humid climate (average relative humidity = 76 per cent), it might be expected that the antirachitic wave lengths would be absorbed and their intensity thus considerably diminished in passing through the atmosphere. The mild temperature, however, allows infants and children to be out of doors throughout most of the year. The availability of green vegetables, ample playing space, and light clothing all tend to minimize the incidence of rickets. Williams (1928) and Crawford and Williamson (1930) report that although mild rickets is quite prevalent amongst infants in New Orleans, severe rickets is seldom encountered.

In none of the local studies has there been any attempt to correlate the incidence and severity of rickets with the variations in the antirachitic wave lengths of sunlight, a factor which, in view of our present knowledge, assumes the utmost importance. We have determined the minimum effective antirachitic exposures during the different seasons of the year using rats and chicks, and have correlated these biological data with physical measurements of the solar antirachitic radiation present at the time of

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exposure of the animals. In addition to exposing the animals to direct sunshine and to skyshine (reflected radiation from sky and clouds), we have also exposed parallel groups under Vitaglass, Corex D and Cel-O-Glass.[2]

Rats bred from our stock colony and weighing about 40 grams were divided into groups of 5 or 6 and fed the Steenbock no. 2965 rachitic diet, with distilled water *ad libitum*. Each experiment included a control group in comparison with which the diagnosis was made, for the most part on the basis of the line test and roentgenograms of the femurs and tibias. In some of the early experiments, serum phosphorus determinations were also made. The duration of almost all of the experiments was 5 weeks which is usually sufficient for definite rickets to develop in control groups kept in the animal room, and in only a few instances was it necessary to continue the experiments longer.

In a parallel study, two day old white leghorn chicks of uniform stock were placed in a brooder and fed a diet composed of 57 parts yellow corn, 20 parts powdered skim milk, 20 parts wheat middlings, ½ part salt and 2½ parts ground bone meal. Tap water was given *ad libitum*. When two weeks old, the chicks were weighed, banded and divided into groups of seven to ten each and placed in large cages. Each experiment included two control groups. Two per cent cod liver oil was added to the diet of the first of these and this group was considered as normal. No addition was made to the diet of the second and these birds served as the leg weakness controls. The chicks were weighed weekly and when 8 weeks old were x-rayed and sacrificed, the blood being pooled for serum calcium and phosphorus determinations. Our estimates as to the efficiency of the radiation were based upon a comparison of the general appearance, the growth curves, roentgenograms and calcium and phosphorus analyses with those of the two control groups.

The rats and chicks were kept in adjoining rooms, which were well ventilated, dimly illuminated and of fairly constant temperature. Certain groups of animals were exposed to sunshine and skyshine on the roof of the animal building adjoining the laboratory which is situated on the college campus, about 4 miles away from the business center of the city, at a latitude of 29° 56′ 2″ and about 35 feet above sea level. The atmosphere is reasonably clear of smoke and dust, although the animals were cut off from some of the radiation from the sky by high trees and buildings. The groups exposed to skyshine were placed on the same roof but in the shadow of the building, receiving radiation from all directions except south. The exposures were begun at 10 a.m. and made daily except on extremely cloudy or rainy days. Consequently in many cases an exposure to sun-

[2] These various screens were kindly presented to us by the manufacturers, Vitaglass Corporation, Corning Glass Works and Acetol Products, Inc., respectively, to whom we here express again our thanks.

shine means that sunshine was available for a definite percentage of this time, while for the remainder, the animals received skyshine or reflected energy from sky and clouds. Several pieces of each of the screens used (Vitaglass, Corex D and Cel-O-Glass) were measured for cut-off and transmission before being exposed and at intervals later. They showed no effect of solarization after 15 months of use, undoubtedly due to the fact that the total time for which they were exposed was relatively short.

The measurement of the solar and sky radiation was done on a tower built on the roof of the animal house adjacent to where the rats were being exposed. Such measurements were begun in 1928 and are still being continued. The extensive information we have thus obtained as to the variation in the total energy (intensity) and the distribution (quality) of sunshine and skyshine in New Orleans will be reported in detail elsewhere, only the pertinent data being referred to here. Spectrograms of the sun were made with a Zeiss spectrograph. The intensity of the total energy of direct sunlight was determined by means of the pyrheliometer described by Gorczynski (1924), the recording millivoltmeter of which was supplemented by a high sensitivity Leeds and Northrup galvanometer. To obtain the distribution of the energy, screens with various cut-offs were used. The intensity of the antirachitic radiation was measured by interposing in front of the thermopile a piece of window glass with a transmission of 0.5 per cent at 313 mμ, 49 to 50 per cent at 334 mμ, 85 per cent at 365 mμ and about 90 per cent in the visible and near infra red. The long wave limit of the ultra violet cut-off is taken at (and including) 313 mμ (instead of 310 mμ, the absolute cut-off) because it is a definite and easily obtained emission line (of the mercury arc spectrum) and, also, because antirachitic action terminates practically at this wave length.

Exposure to sunshine. A total of thirty-three experiments was made on rats, extending from May 31, 1929 to December 26, 1931, the results of which are shown in table 1. As mentioned above, when animals are exposed to sunshine they also receive a large amount of radiant energy from the hemispherical dome of the sky; when it is cloudy the ratio of this skyshine to direct sunshine is high. We have indicated the total and average times of exposure to sunshine (sun + sky, columns 4 and 6) and to skyshine alone, that is to say, when the sun was obscured by clouds (sky, columns 5 and 7). During particularly cloudy months, the rats actually received more skyshine than sunshine, as in experiments 2, 19, 20, 21, 27, 28 and 30. The discrepancies in the sums of the average values (columns 6 and 7) and the daily exposures (column 3) are due to the variation in the number of rainy or very cloudy days.

A daily exposure of from 10 to 30 minutes (average 2.7 to 14.0 minutes) to sunshine and skyshine was more than adequately protective in the first eight experiments, extending from May 31, 1929, through February 14,

TABLE 1

Exposure of rats to sunshine and skyshine

EXPERIMENT NUMBER	DURATION	DAILY EXPOSURE	TOTAL TIME				AVERAGE DAILY EXPOSURE		AVERAGE ENERGY VALUE, DAILY EXPOSURE	DIAGNOSIS
			Sun + Sky		Sky		Sun + Sky	Sky		
	1929	*minutes*	*hours*	*minutes*	*hours*	*minutes*	*minutes*	*minutes*	*gm. cal./cm.²*	
1	May 31–July 6	10	1	40	1	10	2.7	2.0	0.00119	–
2	July 24–Aug. 29	30	5	35	9		9.5	15.0	0.00548	–
3	Sept. 26–Oct. 30	30	9	15	4	45	16.0	8.1	0.00614	–
4	Oct. 6–Nov. 12	10	3	5	1	50	5.1	3.0	0.00270	–
5	Oct. 30–Dec. 4	30	7		5		11.7	8.3	0.00423	–
6a	Nov. 16–Dec. 20	10	3	10	1		5.4	1.7	0.00179	–
6b	Nov. 16–Dec. 20	20	6	20	2		10.8	3.4	0.00358	–
7	Dec. 5–Jan. 17	30	8		6		14.0	10.0	0.00195	–
	1930									
8	Jan. 3–Feb. 14	10	3	10	1	40	4.5	2.4	0.00102	–
9	Feb. 10–Mar. 24	10	2	20	1	20	3.3	1.9	0.00071	4–; 2++
10	Feb. 24–Mar. 31	5		45		30	1.3	0.8	0.00045	3+++; 3++++
12	Mar. 29–May 1	10	3		1	20	5.5	2.4	0.00290	–
14	May 3–June 7	5	1	15		50	2.2	1.4	0.00167	–
15	May 17–June 24	5	1	45		50	2.7	0.8	0.00158	–
17a	June 16–July 21	5	2	10		20	3.7	0.6	0.00217	–
17b	June 16–July 21	5*	1			15	1.7	0.1	0.00099	3–; 2++
19a	July 25–Sept. 4	8	1	20	2	16	1.9	3.3	0.00086	–
19b	July 25–Sept. 4	10	1		1	40	1.4	2.4	0.00064	–
20	Sept. 5–Oct. 10	5	1	5	1	20	1.9	2.1	0.00052	–
21	Nov. 24–Dec. 29	20	3	20	4		5.4	6.7	0.00175	–
	1931									
22	Jan. 5–Feb. 9	10	2	30	1		4.3	1.7	0.00086	2++; 2+++
23	Feb. 19–Mar. 26	15	4	40	1	30	8.0	2.7	0.00308	–
24	Apr. 7–May 12	5	1	20	1		2.3	1.7	0.00096	–
26	May 16–June 20	5	1	30		50	2.6	1.4	0.00115	–
27	June 5–July 11	5	1	5	1	20	1.8	2.2	0.00061	–
28	June 24–July 29	5		55	1	10	1.6	2.0	0.00054	–
29a	July 17–Aug. 20	5*	1			15	1.7	0.4		–
29b	July 17–Aug. 20	5†		40		15	1.1	0.4		–
30	July 31–Sept. 1	5		50	1	15	1.6	2.3		–
31a	Aug. 24–Sept. 26	5	1	10	1	10	2.1	2.1		–
31b	Aug. 24–Sept. 26	5‡		30			0.9	0.0		–
32	Sept. 30–Nov. 4	5	1	25		50	2.4	1.4	0.00090	2–; 3++
33	Nov. 21–Dec. 26	8	1	44		24	2.7	0.7		1–; 5+

* = 3 times each week; † = twice a week; ‡ = on clear days only.

– = no rickets; + = borderline; ++ = mild; +++ = moderate; ++++ = florid rickets.

1930. Since our chief aim was to determine the seasonal variation in the minimum amount of antirachitic radiation in New Orleans sunlight, we accordingly shortened the exposures in the succeeding experiments. The minimum individual exposure time was set at five minutes, and in some cases was obviously longer than necessary. To secure further information on this point, a few groups of rats, while exposed for 5 minutes, were put out only 2 or 3 times a week. An average daily exposure to sunshine (and skyshine) of between 2 and 3 minutes was amply antirachitic from April through October. Of particular interest are experiments 1, 15 and 26 during similar periods (May and June) of 1929, 1930, and 1931, in which protection was afforded by average daily exposures of 2.7, 2.7 and 2.6 minutes, respectively. The minimum effective exposure from November through March was found to be from 5 to 6 minutes, shorter exposures during this period (expts. 9, 10, 22 and 33) being only partially protective. We have included experiments 9, 10 and 23, performed during the latter half of February and March, in this grouping, although the minimum effective time used was 8 minutes (expt. 23). Since 3.3 minutes (expt. 9), however, was almost completely protective, we have taken the mean value as representing the approximate threshold. Note should be made that although an average daily exposure of 1.3 minutes during the latter part of February and March (expt. 10) was followed by severe rickets, an average daily duration of 1.4 minutes was protective from July 25 to September 4 (expt. 19b).

Vitaglass was first used in experiment 7 and in fifteen subsequent experiments, Corex D in eight and Cel-O-Glass in seven experiments. The requisite increase in the duration of the exposures when these screens are used is proportional to the absorption of antirachitic radiation by the screens. For example, with Vitaglass the effective calculated exposure is from 2 to 2.5 times as long as to direct sunlight. The calculated effective exposures through Vitaglass are from 4 to 7.5 minutes for April to October, inclusive, and from 10 to 15 minutes from November to March, inclusive. For Corex D, the values are from 3 to 4 minutes and from 6 to 8 minutes, and for Cel-O-Glass from 7 to 10 and from 17 to 20 minutes, respectively. Our actual findings are in general agreement with these calculated values, thus demonstrating that our average exposures to direct sunshine (without the screens) were fairly close to the minimum durations necessary for the prevention of rickets.

The experiments on chicks were started on October 16, 1929, and terminated on January 2, 1932. The data are given in table 2. They show that average daily exposures of about 2 minutes to sunshine and skyshine during July, August and September; of between 2 and 3 minutes during April, May, June and October; of 4 to 5 minutes during November, December and January; and of 3 to 5 minutes during February and March, will pre-

TABLE 2

Exposure of chicks to sunshine and skyshine

EXPERIMENT NUMBER	DURATION	DAILY EXPOSURE	TOTAL TIME				AVERAGE DAILY EXPOSURE		AVERAGE ENERGY VALUE DAILY EXPOSURE	AVERAGE WEIGHT AT 8 WEEKS	Ca	P	DIAGNOSIS
			Sun + Sky		Sky		Sun + Sky	Sky					
	1929	*minutes*	*hours*	*minutes*	*hours*	*minutes*	*minutes*	*minutes*	*gm. cal./cm.²*	*grams*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
2	Oct. 16–Nov. 27	30	11	30	3		16.0	4.3	0.00397	331	11.10	6.04	–
3	Nov. 6–Dec. 16	10	2	50	2		4.2	3.0	0.00073	302	12.72	5.56	–
4	Dec. 5–Jan. 10	30	6	30	7		11.0	11.6	0.00270	381	9.76	5.00	–
	1930												
5	Jan. 4–Feb. 14	10	3	10	1	30	4.6	2.2	0.00108	331	13.9	6.08	–
6	Feb. 18–Mar. 31	10	2	55	1		4.2	1.4	0.00162	328	11.5	4.85	–
7	Mar. 20–May 9	5	1	50	1	20	2.2	1.6	0.00113	424	13.4	6.12	–
8	Apr. 5–May 23	10	3	50	2	10	4.8	2.7	0.00226	500	13.4	5.64	–
11	Aug. 5–Oct. 1	5	1	50	1	30	1.9	1.6	0.00076	444	13.9	5.28	–
12	Oct. 6–Nov. 15	5	1	30		35	2.3	0.9	0.00069	423	12.7	5.64	–
13	Nov. 18–Dec. 30	5		50	1		1.2	1.4	0.00026	291	11.7	2.84	3–; 3++
	1931												
14	Dec. 31–Feb. 9	7.5	1	30	1	20	2.1	2.0	0.00046	259			2+; 3++; 3+++
15	Feb. 19–Apr. 1	8	2	30		32	3.6	0.8	0.00142	338			–
16	Apr. 7–May 16	5	1	45	1		2.6	1.5	0.00112	350	13.37	5.64	–
17	May 19–June 27	5	1	45		50	2.6	1.2	0.00141	376	13.08	5.44	–
18	July 2–Aug. 13	5	1	25	1	15	2.0	1.8	0.00104	512		5.46	–
19	Sept. 16–Oct. 27	5*	1	10	1		2.4	1.7	0.00090	366	13.5	5.70	–
20	Nov. 18–Jan. 2	8	2	32		24	3.6	0.5		310		4.52	–

* = 4 times a week.

– = no, + = mild, ++ = moderate, +++ = severe leg weakness.

vent the appearance of leg weakness and promote normal growth. Average daily exposures of from 2.0 to 3.8 minutes through Vitaglass or Corex D prevented leg weakness and stimulated normal growth from April through October, and 4.5 to 5.4 minutes was adequate during the rest of the year. The fact that in several experiments the same average daily exposures to sunshine with and without the screens were effective unquestionably indicates that the exposures without the screens were well above the minimum and that shorter average daily exposures would have been ample. The results with Cel-O-Glass show that, even with its relatively poor transmission (27 to 30 per cent), average exposures of from 5 to 6 minutes are adequate in May and June and in September and October, and partially so in January.

TABLE 3

Average amounts of antirachitic radiation during the year at 10 a.m.

DATE	NUMBER OF DAYS MEASURED	GM. CAL. PER SQ. CM. PER MINUTE	μW PER SQ. CM.	NUMBER OF DAYS MEASURED	GM. CAL. PER SQ. CM. PER MINUTE	μW PER SQ. CM.
	1930			*1931*		
Jan	3	0.000252	17.64	6	0.000226	15.82
Feb	5	0.000213	14.91	5	0.000195	13.65
Mar	5	0.000385	26.95	5	0.000401	28.07
Apr	6	0.000528	36.96	3	0.000485	33.95
May	9	0.000563	39.41	4	0.000453	31.71
June	4	0.000836	58.52	4	0.000565	39.55
July				2	0.000634	44.38
Aug	3	0.000572	40.04	1	0.000737	51.59
Sept	3	0.000452	31.64			
Oct	7	0.000373	26.11	4	0.000374	26.18
Nov	6	0.000278	19.46	3	0.000274	19.18
Dec	5	0.000183	12.81			

During the winter months there is very little radiant energy shorter than 313 mμ in New Orleans sunlight except on brilliantly clear days. The average values for the months of the year and the number of determinations from which they were derived are given in table 3. It is to be remembered that these averages were obtained from measurements made on clear days only or on days when the sun was not obscured by clouds. Since there are more cloudy and rainy days than there are clear days in New Orleans (average for 60 years shows only 124 clear days annually), the values given represent the approximate upper limits that may be expected. Coblentz, Stair and Hogue (1931) obtained values ranging from 60 to 80 μw (microwatts) at 10 a.m. during June in Washington and about 57 μw in July. They conclude that the average amount of ultra violet

radiation of wave lengths less than and including 313 mμ for mid-latitude sea level stations, during the clearest weather, during midday in midsummer, amounts to about 0.00129 gm. cal. per sq. cm. per min. (90 μw per sq. cm.) decreasing to about 0.000285 gm. cal. per sq. cm. per min. (20 μw per sq. cm.) on the clearest days in midwinter. Our midsummer, midday values are of the same order, ranging from 65 to 70 μw, although we sometimes obtained values of 38 to 49 μw on clear days in midwinter. The weather for the year 1930 was considered by the local Weather Bureau as remarkably near the normal, or long term mean, and the total number of determinations represents over one-third of the clear days during the year. The differences in the values for similar months in 1930 and 1931 reflect the variations in the amount of haze and clouds present at the time. The single value for August, 1931, was obtained on an unusually clear, dry day and is probably higher than an average of several days would have been, since the weather in New Orleans during July and August is usually very cloudy, the average number of clear days for the last 56 years being only 7 and 8, respectively, as compared with 10 in December and 16 in October. DeBuys (1924) and DeBuys and von Meysenbug (1924) found that the peak of incidence of rickets in infants in New Orleans varied in different years. Thus, in 1922 they found the greatest incidence during March, while in the following year, the peak had shifted to late in July. Our findings for 1930 and 1931 clearly demonstrate that the reason for this variation in time of incidence is the variation in the amount of radiation shorter than 313 mμ.

Spectrograms, taken while the animals were being exposed (9:00–10:30 a.m.), show that at this time of the day the spectrum very rarely extends to 300 mμ and we have only one record on June 6, 1930, of 299 mμ. During the winter months the spectrum seldom extends below 306 mμ except on very clear days after a rain storm when the air has been washed clean. Throughout the year, in fact, there are relatively few days on which between 11 a.m. and 1 p.m. the spectrum extends below 304 mμ. From June 1 to December 31, 1929, only 5 out of 55 days measured showed limits shorter than 304 mμ, only 7 days out of 77 in 1930, and only 15 days out of 50 in 1931. Air pollution, clouds and haze decrease the extent of the spectrum so that even in August for days on end there are no wave lengths shorter than 310 mμ. In many cases there must be considerable carry-over of antirachitic effect from those days when the shorter wave lengths are present (see Russell and Howard, 1931). In experiment 31b (table 1), six 5 minute exposures on clear days over a period of 5 weeks protected a group of rats as adequately as did a 5 minute daily exposure of another group for the same length of time. Since solar radiation of the most effective antirachitic wave lengths, 302 mμ and shorter, is so seldom present in this city, and since it is possible at all times of the year to prevent

rickets in rats and chicks by exposing them to the available sunshine, the conclusion is obvious that the effects are being exerted, for the most part, by wave lengths between 304 and 313 mμ.

The smallest amount of solar energy of wave lengths less than 313 mμ protective for the rats (see table 1) was an average of 0.00052 gm. cal. per sq. cm. daily (expt. 20), a total of 0.0182 gm. cal. per sq. cm. The usual shortest wave length present in the sunshine during this period was 304 mμ or longer, the spectrum extending to 301 mμ on one of the days measured. Such values do not represent all of the radiation which the animals received. The pyrheliometer with which they were obtained measures only the direct solar radiation, giving no information as to the energy in the diffusely reflected radiation from the sky and clouds which the animals also receive. Our results show that an average daily exposure of rats to somewhat less than 0.001 gm. cal. per sq. cm. of wave lengths shorter than 313 mμ was partially or completely antirachitic from April through October, while exposures of from 0.001 to 0.002 gm. cal. per sq. cm. were effective during the remainder of the year. The minimum amount of energy effective in preventing leg weakness and in promoting growth in the chicks was 0.00069 gm. cal. per sq. cm. daily, or a total of 0.0290 gm. cal. per sq. cm. The next smallest amount of energy which was completely adequate for calcification and growth was 0.0378 gm. cal. per sq. cm. and in every case where amounts greater than this were used there was normal development of the chicks.

The effective exposures to sunshine which we used are shorter than any that have been published. Day (1932) reported from Little Rock, Arkansas, that the average daily amount of sunshine necessary to afford the same partial protection against rickets in rats receiving a rachitogenic diet varied from 5 minutes in May, June and July to 168 minutes in December, with intermediate values for the other months. Stein and Lewis (1931) at Denver, during June and July, 1927, found an average daily exposure to sunshine of 1.957 minutes inadequate, of 3.75 minutes partially and longer than 7.5 minutes more or less completely protective. Lewis, Frumess and Stein (1931) failed to find a seasonal variation in antirachitic efficiency in Denver because, as they claim, a certain minimum amount of the antirachitic wave lengths reaches the earth throughout the year, owing to the high percentage of sunshine, the low humidity and the small amount of smoke in the atmosphere. Godfrey (1931) also reported the lack of any seasonal variation in solar antirachitic efficiency in Honolulu. Sloan (1929), in a study of the seasonal variation of the solar antirachitic radiation in Ithaca, N. Y. (latitude 42° 30′ N, 283 M) found that 30 to 40 minute daily exposures of chicks under Corex A at high noon were about threshold for the prevention of leg weakness during the winter, 10 minutes in the spring, and 2.5 to 5 minutes not quite adequate in the

summer. There is still considerable difference between his findings and ours, especially during the winter months, even after reducing his values by 10 to 15 per cent to correct for the transmission loss through Corex A.

The relatively high intensity of the available radiation in New Orleans throughout the year undoubtedly accounts for the short exposures necessary to prevent rickets. Tisdall and Brown (1929, 1931) report that there is a marked increase in the antirachitic effect of sunshine in Toronto when the sun reaches a midday altitude of 35° late in February and a marked decrease at about the same altitude in October. The increased antirachitic effect with the sun at 35° is due to a general increase in the intensity of the rays ranging from 320 to 294 mμ rather than to the presence of shorter rays which do not get through with the sun at the lower altitudes. Dick (1922) showed that severe rickets was rare, or absent, in all places where the minimal seasonal altitude of the sun was above 35°, and Tisdall and Brown believe that severe rickets occurs in those places where the altitude of the sun is below 35° for several months of the year. The minimal seasonal midday altitude of New Orleans is 37° so that one would expect sufficient antirachitic radiation present at all times of the year. Our findings accord with this supposition and show that the seasonal variation which we observed was in great measure due to the fact that the solar altitude in New Orleans during November, December and January at 10 a.m. (at which time the animals were exposed) is 32°, 29° and 32°, respectively, and above 35° from February through October. Tisdall and Brown (1931) believe that the reason why Lewis, Frumess and Stein did not find a seasonal variation in solar antirachitic radiation was probably due to the fact that their experiments were done in February, March and April, at which time the sun in Denver has already reached an altitude of 35° and therefore has a high antirachitic potency. Taking into account the latitude and altitude (1650 M) of Denver, Tisdall and Brown would expect to find in that city a low antirachitic effect of sunshine only from December 1 to January 15. The difference between the latitude of Ithaca (42° 30′ N) and of New Orleans (29° 56′ N) accounts in some measure for the difference in the duration of minimal effective exposures in Sloan's study and in ours, since the minimal seasonal midday altitude of the sun in Ithaca is only 24°, rising to about 37° in February and to a maximum of 71° in June. The midday altitude of the sun in New Orleans at this time is about 83°.

Exposure to skyshine. The term *skyshine* is used to designate the sun's rays that are reflected from the sky and clouds, in contradistinction to the rays received from the sun itself. It is obvious that animals or infants exposed to sunshine also receive skyshine. The importance of skyshine as a source of ultra violet radiation was first pointed out by Dorno (1919). He showed that at Davos (altitude 1560 M), even under the best conditions and with a solar altitude of 60°, the ultra violet radiation of wave lengths

less than 366 mμ in direct sunshine was only 90 per cent of that in skyshine. With lower solar altitudes the ultra violet in skyshine increases markedly, so that with a solar altitude of 30°, sunshine contains only about 30 per cent as much ultra violet as skyshine. Our own measurements during the month of July, 1931, show that in New Orleans at 10 a.m., with a solar altitude of about 61°, the intensity of the ultra violet of wave lengths less than 313 mμ is about 20 per cent greater in skyshine than in direct sunshine.

Our experiments were designed to determine the minimal exposures to skyshine throughout the year which would prevent rickets and to compare these results with those obtained on exposure to sunshine and skyshine. The methods have been outlined above. The same screens were used as in the sunshine experiments. The results of exposure of rats to skyshine are given in table 4. An average daily exposure to skyshine of from 16 to 17 minutes is close to the minimum effective amount which will prevent rickets in rats in New Orleans from May through September, although somewhat shorter exposures during June, July and August, 1931, werep rotective. Thus the antirachitic action of skyshine is at least one-sixth as potent as that of sunshine, including skyshine, during these months.

Average daily exposures to skyshine of from 22 to 24 minutes (about 4 times those of sunshine + skyshine) were adequate during October, November and December. The exposures of 67 and 44.6 minutes used in January (expts. 8 and 22) were unquestionably too long. Judging from the average exposures found adequate in December and February, an average daily exposure of from 22 to 28 minutes would have been effective. The results for February and March, 1930 and 1931, vary. Thus an average daily exposure of 28 minutes from February 10 to March 24, 1930 (expt. 9) was not quite sufficient, although an average daily exposure of 16 minutes from February 19 to March 26, 1931 (expt. 23) was protective. Similar durations of exposure (15 and 16 minutes) during March of 1930 and 1931 (expts. 10 and 23) resulted in florid rickets in the former, and no rickets in the latter case. The effective exposures of 47 minutes (expt. 12) and 25 minutes (expt. 24) during April were probably longer than necessary. We may conclude that an average daily exposure of somewhat less than 28 minutes would be effective in February and that exposures of from 16 to 20 minutes are protective in March and April.

Partial or complete protection was afforded to rats exposed daily to skyshine under Vitaglass from May through September for average periods of from 28.8 to 50 minutes. This was to have been expected from the transmission data of this screen. Similarly from October through January average daily exposures of from 51.1 to 67 minutes were antirachitic. The results for February, March and April for 1930 and 1931 differ, as did the results on direct skyshine, although the results with and without the screen

are consistent for each year. Exposures of from 28.3 to 50 minutes were adequate in 1931, but not so in 1930. This would indicate that the differences obtained were due to variations in the amounts of antirachitic radiation present at these times in the two years.

TABLE 4

Exposure of rats to skyshine

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	DAILY EXPOSURE	TOTAL TIME		AVERAGE DAILY EXPOSURE	DIAGNOSIS
	1929	*minutes*	*hours*	*minutes*	*minutes*	
1	May 31–July 6	10	4	50	8.3	++
2a	June 24–July 29	30	14	5	24.0	−
2b	June 24–July 29	60	28	10	48.0	−
3	Sept. 26–Oct. 30	180	81	35	140.0	−
4	Oct. 8–Nov. 12	30	14	20	24.0	−
5	Oct. 30–Dec. 4	180	72		120.0	−
6a	Nov. 16–Dec. 20	30	13		22.0	−
6b	Nov. 16–Dec. 20	90	36	30	63.0	−
7	Dec. 5–Jan. 17	180	84		148.0	−
	1930					
8	Jan. 3–Feb. 14	90	47	50	67.0	−
9	Feb. 10–Mar. 24	60	20		28.0	4−; 2+
10	Feb. 24–Mar. 31	30	8	30	15.0	++++
12	Mar. 29–May 1	60	26		47.0	−
14	May 3–June 7	30	12	30	22.0	−
15	May 17–June 24	20	9		14.0	1−; 2++; 2+++
17	June 16–July 21	20	9	40	16.0	1−; 2++; 2+++
19	July 25–Sept. 4	20	9	20	13.6	++
21	Nov. 24–Dec. 30	60	23		35.0	−
	1931					
22	Jan. 5–Feb. 9	60	26		44.6	−
23	Feb. 19–Mar. 26	45	11		16.0	−
24	April 7–May 12	30	14	30	25.0	−
26	May 16–June 20	20	9	20	16.0	−
27	June 5–July 11	15	7	15	11.8	−
28	June 24–July 29	20	9		15.3	−
29	July 17–Aug. 20	15	7	15	12.4	−
30	July 31–Sept. 1	20	8	20	15.5	−
31	Aug. 24–Sept. 26	20	9	20	16.5	−
32	Sept. 30–Nov. 4	20	9		15.4	3++; 2+++
33	Nov. 21–Dec. 26	30	8		13.3	3+; 2++; 1+++

Rats exposed to skyshine under Corex D from June through September obtained almost complete protection with average daily exposures of from 23 to 28 minutes, and average exposures of from 40 to 50 minutes were protective from January through April. The results with Cel-O-Glass show that sufficient protection was afforded during June and July with

average daily exposures of from 23 to 48 minutes, but that relatively long exposures (up to 94 minutes) were inadequate at other times of the year.

The data on exposure of chicks to skyshine are given in table 5. An average daily exposure of between 6 and 11.4 minutes from July through October; of 35 minutes from November through February; and of 18 to 19 minutes from March through June is ample for proper calcification and growth. The latter two values are the means of the actual amounts used

TABLE 5

Exposure of chicks to skyshine

EXPERIMENT NUMBER	DURATION	DAILY EXPOSURE	TOTAL TIME		AVERAGE DAILY EXPOSURE	AVERAGE WEIGHT AT 8 WEEKS	Ca	P	DIAGNOSIS
	1929	*minutes*	*hours*	*minutes*	*minutes*	*grams*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
2	Oct. 16–Nov. 27	180	89	40	127	342	12.86	5.44	–
3	Nov. 6–Dec. 16	90	42	30	64	345	13.60	5.16	–
4	Dec. 5–Jan. 10	180	69		113	410	10.72	5.48	–
	1930								
5	Jan. 4–Feb. 14	90	42		61	330		5.72	–
6	Feb. 18–Mar. 31	60	19		28	329	10.50	4.92	–
7	Mar. 20–May 9	30	19		23	369	13.40	5.84	–
8	Apr. 5–May 23	60	36		45	361	12.30	4.95	–
11	Aug. 5–Oct. 1	30	19		20	511	11.87	5.88	–
12	Oct. 6–Nov. 15	15	4		6	443	12.0	5.75	–
13	Nov. 18–Dec. 30	10	4		5.9	253		3.30	3++; 4+++
	1931								
14	Dec. 31–Feb. 9	12.5	4	55	7	180			+++
15	Feb. 19–Apr. 1	15	6	45	9.6	259			5–; 1++
16	Apr. 7–May 16	15	8	15	12.4	315	12.31	3.48	1–; 4++; 2+++
17	May 19–June 27	20	10	40	15.6	372	13.32	5.08	5–; 2++
18	July 2–Aug. 13	15	6		11.4	468		5.16	–
19	Sept. 16–Oct. 27	10	5	30	9.4	380	10.1	6.10	–
20	Nov. 18–Jan. 2	20	7	20	10.5	278		4.72	3–; 3++

and represent an approximation of the effective average exposures. Skyshine was thus about one-fourth to one-fifth as effective as was sunshine and skyshine from July through October, and about one-sixth to one-seventh as effective during the rest of the year. Chicks exposed to skyshine under Vitaglass and Corex D require an average exposure of from 11.4 to 14.0 minutes from July through October, from 50 to 60 minutes from November through February, and from 25 to 30 minutes from March through June, for the prevention of leg weakness and stimulation of growth.

The experiments with Cel-O-Glass are of interest because of its extensive use by poultrymen. Complete protection and good growth were obtained in September and October (expt. 19) with an average exposure to skyshine of 28.3 minutes and with 39 minutes during May and June (expt. 17), and even in January, an average daily exposure of 20.3 minutes was partially protective.

In the discussion of the energy equivalents of the sunshine exposures, emphasis was placed on the fact that the values given represented only the amount of direct solar radiation and did not include the diffuse energy received from sky and clouds. During the months of June and July, 1931, we used an Eppley pyrheliometer, the sensitive area of which is exposed, not only to direct solar radiation, but also to that diffusely reflected from the sky and clouds. This instrument, a modification of that designed by Kimball and Hobbs (1923), is thus exposed to radiation similar to that received by animals exposed to "sunshine." By shielding the thermopile from the direct solar rays it is possible to measure the intensity of skyshine, and thus determine the part it plays in furnishing antirachitic energy.

Application of these data to the experiments in progress during these two months shows that the rats in experiment 27 (table 1) received an average total radiation (sunshine + skyshine), of wave lengths shorter than 313 mμ, equivalent to 0.000407 gm. cal. per sq. cm. per min., or 0.00073 gm. cal. per sq. cm. daily, of which 0.00061 gm. cal. per sq. cm. was direct sunshine. In addition (see table 1) the animals received an average of 2.2 minutes of skyshine daily (in the absence of direct sunshine). The average total radiation of less than 313 mμ in skyshine for this period was 0.000290 gm. cal. per sq. cm. per min. The sum of these values, the total amount of antirachitic radiation which the rats received, was therefore 0.00137 gm. cal. per sq. cm. daily. The skyshine group in this series received an average daily exposure of 11.8 minutes (table 4) which is equivalent to 0.0034 gm. cal. per sq. cm. of wave lengths less than 313 mμ, Similar treatment of the data for experiment 28 shows that the animals in the sunshine group received a total of 0.0012 gm. cal. per sq. cm. daily of wave lengths less than 313 mμ as compared to the skyshine group which received 0.0037 gm. cal. per sq. cm. daily. Similarly, the sunshine (sunshine + skyshine) group of chicks exposed during this period (expt. 18, table 2) was receiving radiation, of wave lengths shorter than 313 mμ. equivalent to 0.000449 gm. cal. per sq. cm. per min., or 0.00090 gm. cal. per sq. cm. daily, from the sun and the sky, and in addition an average of 1.8 minutes daily of skyshine radiation alone, equivalent to 0.000244 gm. cal. per sq. cm. per min., a total of 0.00134 gm. cal. per sq. cm. daily. In this experiment the group exposed to skyshine received a total of 0.00289 gm. cal. per sq. cm. daily. It is quite evident that the exposures to sky-

shine in each case could have been reduced by at least one-half and would still have been adequate.

These calculations indicate that antirachitic energy equivalent to a total of from 0.0420 to 0.0490 gm. cal. per sq. cm. (0.0012 to 0.0014 gm. cal. per sq. cm. daily for 35 days) can be considered as the amount which will prevent the appearance of rickets in rats, while 0.0563 gm. cal. per sq. cm. (0.00134 gm. cal. per sq. cm. for 42 days) is more than adequate protection for chicks. That the value of 0.0490 gm. cal. per sq. cm. represents the approximate minimal energy requirement for rats when no wave lengths shorter than 300 mμ are present is shown by additional experiments in which rats were exposed to this and to smaller amounts of radiation from a flaming carbon arc (25–28 A; 55–60 V; Sunshine Carbons) and from a quartz mercury vapor lamp (5–6 A; 70–80 V).

Three sets of experiments were performed in which the rats were exposed 1, to the unscreened radiation of the arcs; 2, to radiation longer than 290 mμ, the arcs being screened by a Corex D filter; 3, to radiation longer than

TABLE 6

Antirachitic energy content of flaming carbon and quartz mercury vapor arcs at 1 M, in gm. cal. per sq. cm. per min.

	SHORTER THAN 319 mμ	290–319 mμ	300–319 mμ
Pan Ray Arc 25–28 A; 55–60 V; Sunshine Carbons	0.00359	0.00213	0.00142
Quartz Hg Vapor Arc 5–6 A; 70–80 V	0.00427	0.00221	0.00122

300 mμ, Pyrex glass being used as a filter. The wave lengths included in the second set represent those found in sunshine under ideal conditions, 290 mμ being considered the approximate wave length limit of solar radiation, while the wave lengths included in the third set are those previously indicated as having been present under the conditions of our experiments. The antirachitic energy content and distribution of the two sources was measured spectroradiometrically with a Hilger monochromatic illuminator (table 6) and the rats exposed weekly for lengths of time so calculated (proper corrections being made for the percentage wave length transmission of the filters used) as to provide a total radiation of 0.0490 gm. cal. per sq. cm. over a period of five weeks.

Complete protection was obtained with this amount of energy in the first two sets of experiments in which the rats were exposed to the unfiltered radiation of the carbon and quartz mercury vapor arcs and to the radiation filtered through Corex D. Radiation equivalent to one half this amount prevented rickets in the groups exposed to the quartz mercury vapor arc, but only partially protected those exposed to the carbon arc. In the third

set of experiments in which the rats were irradiated through Pyrex glass (in the absence of wave lengths shorter than 300 mμ), those exposed to the carbon arc radiation showed no rickets, while several of the animals exposed to the quartz mercury vapor lamp showed mild rickets. Shorter exposures resulted in only partial or no protection in both groups. Differences in the spectra of the two arcs account for the variation in the results, the energy in the carbon arc being chiefly of wave lengths longer than 300 mμ, while the mercury vapor lamp has the powerful antirachitic band at 297 to 302 mμ but relatively little effective antirachitic energy of longer wave lengths, the line at 313 mμ being only slightly antirachitic.

SUMMARY

Exposure of rats and chicks on rickets-producing diets over periods of 31 and 27 months, respectively, shows that an average daily exposure to New Orleans sunshine (sun + sky) of from 2 to 3 minutes from April through October prevents the appearance of rickets, as do average daily exposures of from 5 to 6 minutes from November through January. Average daily exposures of chicks for from 3 to 5 minutes, and of rats for from 5 to 6 minutes are antirachitic during February and March.

Average daily exposures to skyshine of from 16 to 17 minutes from May through September prevent rickets in rats, as do average daily exposures from 22 to 28 minutes from October through February, and from 16 to 20 minutes during March and April. Proper calcification and growth is obtained in chicks exposed to skyshine with average daily exposures of from 6 to 11.4 minutes from July through October; of 35 minutes from November through February; and of from 18 to 19 minutes from March through June. Rats and chicks exposed under Vitaglass, Corex D and Cel-O-Glass to sunshine (sunshine + skyshine) or to skyshine are protected throughout the year by durations of exposure correspondingly greater in proportion to their relative absorption.

The average daily amount of direct solar radiation of wave lengths shorter than 313 mμ which will prevent rickets in rats is equivalent to less than 0.001 gm. cal. per sq. cm. (70 μw) from April to October, and between 0.001 and 0.002 gm. cal. per sq. cm. during the rest of the year. The minimum amount of solar energy of wave lengths shorter than 313 mμ, effective in preventing leg weakness and promoting growth in chicks was found to be about 0.00069 gm. cal. per sq. cm. (48.3 μw) daily. Measurement of the total antirachitic radiation, which includes the additional skyshine to which the animals are exposed, shows that in June and July antirachitic radiation from sunshine and skyshine equivalent to between 0.042 and 0.049 gm. cal. per sq. cm. (0.0012 (84 μw) to 0.0014 (98 μw) gm. cal. per sq. cm. daily for 35 days) will prevent rickets in rats and a total of 0.0563 gm. cal. per sq. cm. (0.00134 gm. cal. per sq. cm. for 42 days) will provide for normal calcification and growth of chicks.

Experiments in which rats were exposed to carbon and quartz mercury vapor arc radiation equivalent to 0.049 gm. cal. per sq. cm. demonstrate that this value can be considered as the approximate minimum amount of energy needed to prevent rickets in rats when no wave lengths shorter than 300 mμ are present, and affords, therefore, more than adequate protection when shorter wave lengths are present.

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ALTERATIONS IN BLOOD LACTIC ACID AS A RESULT OF EXPOSURE TO HIGH OXYGEN PRESSURE

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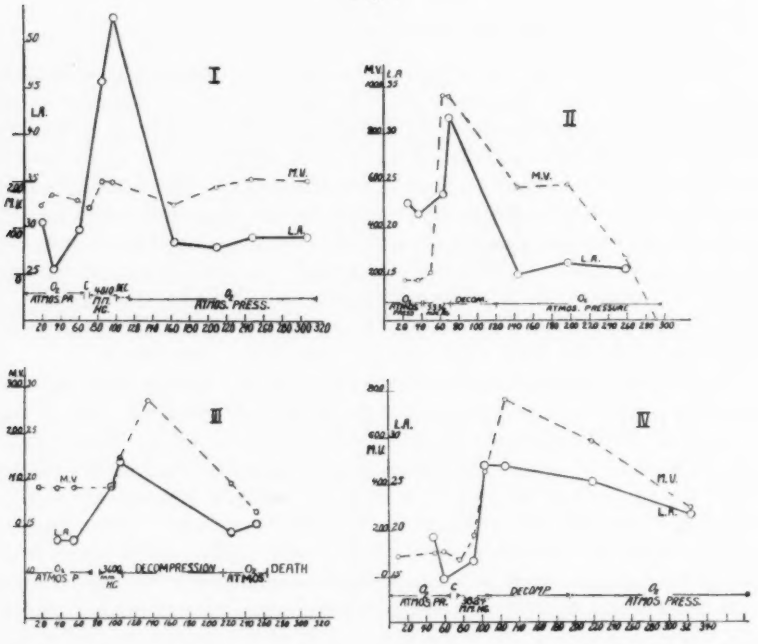
The removal of lactic acid from the mammalian organism is essentially an aerobic process. We might suppose then that in the presence of an abundance of oxygen the lactic acid content of the organism would be at a minimum. There is evidence however that with exposure of an animal to high oxygen pressures, oxidations are diminished. Might this diminution be reflected in the lactic acid content of the organism? The present series of experiments was carried out to determine the alterations in the lactic acid content of the blood of animals exposed to several atmospheres of oxygen. It is of course understood that the changes in the blood lactic acid do not necessarily constitute an infallible criterion of the changes in the total lactic acid content of the organism.

The animals used in our experiments were dogs weighing from eight to twelve kilograms, anesthetized with morphine and urethane. In all but a few of the experiments anti-coagulant (heparin) was used. The pressure chamber with the accessory apparatus was that described earlier (Bean, 1931). It provided means of recording graphically pulmonary ventilation, blood pressure and pulse rate. Blood lactic acid was determined by the Friedemann, Cotonio and Schaffer method (1927). The blood from which the samples were taken circulated continuously through a glass tube connecting the central and peripheral ends of one carotid artery. This tube in a part of its course ran outside of the pressure chamber and was there provided with a stopcock through which the blood samples were taken with the aid of a syringe. Intravenous injection with the animal under pressure was made possible by connecting the lower end of a burette to a stopcock through the chamber wall and to the femoral vein; the upper end was led back into the chamber by pressure tubing and a second stopcock.

The experimental animal first breathed pure oxygen at atmospheric pressure for about thirty or forty minutes. The pressure was then rapidly raised to about five atmospheres and maintained at this level for approximately twenty minutes. Decompression was carried out in stages and over a period of such duration as to avoid effervescence which might

result from too rapid decompression. Following decompression the animal breathed oxygen at atmospheric pressure to the end of the experiment. Blood samples for lactic acid determinations were taken before, during and after exposure to the high pressure. These samples in most instances were twelve cubic centimeters. In order to obviate so far as possible any deleterious effects which the loss of blood might entail, an equal volume of saline was injected intravenously after the withdrawal of each sample.

Fig. 1



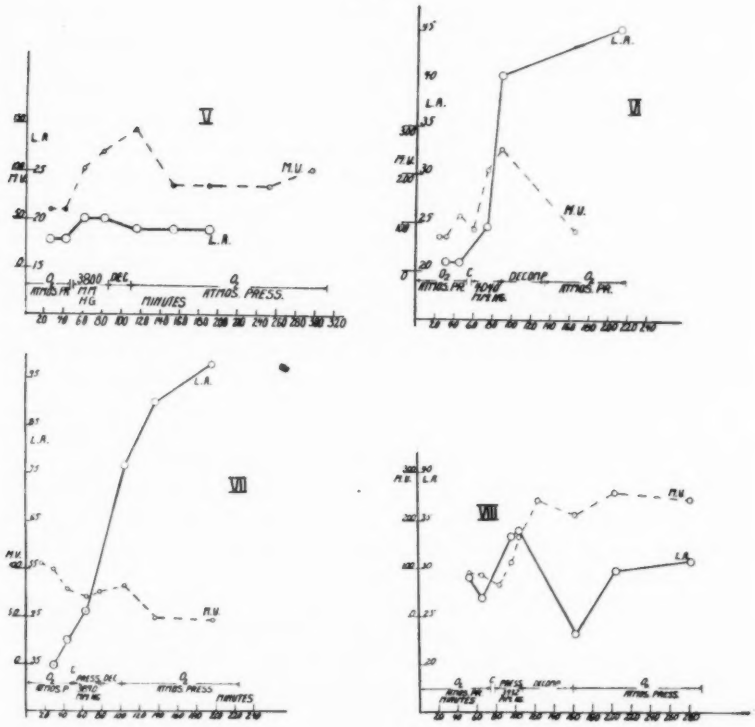
The M. V. figures on the ordinate represent one-tenth the total respiratory minute volume in cubic centimeters.

Breathing pure oxygen at one atmopshere was associated with little or no change in the lactic acid content of the blood. The change if any was usually a decrease. During the periods of exposure to high pressure the blood samples in fifteen of the nineteen experiments constituting this series showed an increase in lactic acid and in four they showed no appreciable change. Of those experiments in which there occurred an increase in the blood lactic acid, six showed complete recovery in so far as lactic acid was concerned, during, or subsequent to decompression. In six others recovery

was evident but incomplete and in the remaining three the blood lactic acid continued to rise through the decompression period and to the death of the animal.

The respiratory minute volume increased during exposure to the high pressure in all the experiments except one, in which there occurred a slight decrease.

Fig. 2



The M. V. figures on the ordinate represent one-tenth the total respiratory minute volume in cubic centimeters.

These changes are illustrated in figures 1 and 2, in which blood lactic acid in milligrams per cent and respiratory minute volume are plotted on the ordinates against time in minutes on the abscissae. The duration of the periods of exposure to the various pressures are indicated along the abscissae. Curve I shows an initial drop in lactic acid during exposure to oxygen at one atmosphere and a return to the original value before

compression was begun. While under an oxygen pressure of 4000 mm. Hg the lactic acid rose from 30 to 54 mgm. per cent; with decompression it returned to the precompression value. Curve II also indicates a preliminary decrease in the blood lactic acid but in this case it approaches the limit of experimental error. During exposure to the increased pressure, which in this instance was 3336 mm. Hg the lactic acid rose appreciably. On decompression and subsequent breathing of oxygen at one atmosphere the lactic acid content of the blood fell to values decidedly below those of the precompression period. An initial drop in blood lactic acid is shown also in curve VIII, figure 2, although it subsequently rose above the precompression level during exposure to oxygen at a pressure of 3932 mm. Hg. A recovery decrease in lactic acid was well marked in this experiment but not sustained. In curve III the initial fall is absent but the increase under pressure and subsequent complete recovery are present as in curves I and II. Curve IV of figure 1 illustrates another experiment in which there occurred an initial fall in the blood lactic acid while the animal was breathing oxygen at one atmosphere pressure and a rise during the high pressure period. The recovery however was neither so complete nor as rapid as in the aforementioned curves. In curve V there is a definite though less pronounced increase in blood lactic acid. Curve VI is illustrative of those experiments in which the lactic acid of the blood continued to rise even after decompression. Curve VII represents an exception in that breathing oxygen at one atmosphere was attended by an increase in the lactic acid of the blood. There was moreover no indication of any recovery from the lactic acid increase after decompression. This experiment was exceptional also in that the respiratory minute volume decreased generally throughout the experiment. It may have been that the animal was in poor condition to begin with, although the blood pressure and the pulse rate did not indicate that such was the case.

The changes in respiratory minute volume consequent upon exposure to high pressures of oxygen used in our experiments are evident from the graphs of figures 1 and 2 (the broken line curves). That breathing oxygen at one atmosphere gives rise to little or no change in the respiratory minute volume finds substantiation in our curves. The increase in ventilation which is attendant upon exposure to high pressures of oxygen is what might be expected considering the earlier findings in this respect of Bert (1878), Smith (1899), Hill and Macleod (1903), Gesell (1923), and Bean (1929). An explanation of this typical respiratory response in oxygen poisoning, based on a broken coördination of the dual function of hemoglobin, has been given by Gesell (1923), and supported by experimental evidence (Bean, 1931). The increase in ventilation was well marked in most of our experiments even though the duration of exposure to high pressure was relatively short.

DISCUSSION. The lactic acid increase in these experiments might conceivably have been due to any one or several of a number of factors which are involved in its metabolism and its shift within the organism.

It has been well established that there is a large increase in lactic acid during severe muscular exercise. At first sight the curves in figures 1 and 2 might suggest that in our experiments the blood lactic acid increase was due to an augmented activity of the respiratory muscles during exposure to the increased oxygen pressure. That this could not have been the primary cause for the increased blood lactic acid may be concluded from a close inspection of these curves. During the breathing of oxygen at one atmosphere (curve I, fig. 1) there is an inverse relationship between the respiratory minute volume and the blood lactic acid. Moreover the relatively small increase in the respiratory movements during exposure to 4000 mm. Hg pressure would in itself be insufficient to cause the attendant large increase in the blood lactic acid. In curve III of the same figure it is to be noted that while the lactic acid content of the blood increased from 12 to 20 mgm. per cent for the first sample taken under the increased oxygen pressure, the respiratory minute volume remained unaltered. In other words, the lactic acid increase preceded the respiratory minute volume increase. Again, in curve IV, figure 1, there is to be seen an inverse relationship between the lactic acid content of the blood and ventilation during the initial period of breathing oxygen at atmospheric pressure. In curve V, figure 2, the ventilation continued to increase while the blood lactic acid decreased on decompression. These instances of a lack of parallelism between blood lactic acid and ventilation support the belief that the rise in lactic acid was not due primarily to increased muscular movement. Moreover there are other conditions which bring about marked hyperpnea without any accompanying increase in the blood lactic acid. Administration of carbon dioxide gives rise to increased activity of the respiratory muscles but the lactic acid content of the blood is decreased. (Gesell, Krueger, Gorham, and Bernthal, 1930.)

Several experiments were performed to determine whether heparin as used in our procedure had any effect on blood lactic acid. Essentially the same results were obtained with and without the use of heparin from which it was concluded that heparin was not a determining factor in the increase in blood lactic acid in our experiments.

The accumulation of blood lactic acid might have been due to its inadequate removal. This removal, barring elimination by way of the skin and kidneys, is an aerobic process. During exposure to high pressures of oxygen, as used in our experiments, the blood is saturated with oxygen (Bean, 1931). Any failure in the removal of lactic acid cannot therefore be attributed to a lack of oxygen. It is possible, however, that even in the presence of an abundance of oxygen, the oxidative mechanisms of the

tissues, notably the liver, which remove lactic acid from the blood might be impaired as a result of oxygen poisoning. In numerous pathological conditions involving injury of the liver cells such as phosphorus poisoning, carcinoma, fatty degeneration and acute yellow atrophy there is a concomitant increase in blood lactic acid. (Büttner, 1926; Valentin, 1925; Noah, 1927; Schumacher, 1926.)

There is evidence that in oxygen poisoning there is a decrease in oxidations (Hill and Macleod, 1903; Bean, 1931). It would appear therefore that the accumulation of lactic acid under high oxygen pressure might be due to a decrease in the oxidative removal rather than to an increase in the rate of production of lactic acid. In this connection it is of interest to note that following exposure to high oxygen pressure a temporary (possibly compensatory) overshooting of oxygen absorption was usually observed (Bean, 1931). The conditions produced by oxygen poisoning might be similar to those resulting from injection of cyanide. In animals injected with sodium cyanide there is every reason to believe that the blood is fully oxygenated yet oxidations decreased and lactic acid increased in the blood. Here with uniform ventilation there was also a tendency toward overshooting of oxygen consumption during recovery. (Gesell, Krueger, Gorham and Bernthal, 1930.)

In considering the increase in blood lactic acid another factor should be taken into account, namely, a broken coördination of the dual function of hemoglobin, which occurs at pressures of oxygen above three atmospheres (Bean, 1929). Under these pressures the tissues obtaining plenty of oxygen for their immediate needs from that in solution in the plasma leave the hemoglobin unreduced. As a result no base is supplied for the transport of carbon dioxide to the lungs. The carbon dioxide accumulates in the tissues turning them acid and this increased acidity is shortly reflected in the blood. Campbell (1930) has found an accumulation of carbon dioxide in tissues of animals exposed to high oxygen pressure. Increased acidity inhibits oxidations. It is possible therefore that in oxygen poisoning the decreased oxygen absorption is due in part at least to the increase in acidity arising from the accumulation of carbon dioxide. The lactic acid accumulation might in turn be attributed to the lowered oxidations. This increase in lactic acid content of the blood would emphasize the acidosis. In this connection it is to be noted that it is not uncommon for the hyperpnea resulting from exposures to high pressures of oxygen to diminish quite abruptly to the point of apnea. This response might be explained as being due to a rapid fatigue of the respiratory control mechanism under the influence of the intracellular acidity which is increasing rapidly, not alone because of the accumulation of carbon dioxide but also because of fixed acids in the centers.

There are however numerous examples in which the relation of acidity,

oxidations and lactic acid content of the blood as suggested above does not exist. It has been found that intravenous injections of sodium bicarbonate give rise to an increase in blood lactic acid (Macleod and Hoover, 1917; Anrep and Cannon, 1923; Gesell, Krueger, Gorham and Bernthal, 1930b) and at the same time an increase in oxygen consumption. Intravenous injection of hydrochloric acid increased the hydrogen ion concentration of the blood, decreased the blood carbon dioxide capacity and the blood lactic acid. Administration of gas mixtures with a high carbon dioxide content (11.42 per cent) diminished oxidations and commonly lowered blood lactic acid. From the results of these experiments in which carbon dioxide was administered with constant artificial ventilation it was concluded that "it would thus appear that neither rate of oxidations nor blood and tissue acidity were the primary determining factors controlling the lactic acid equilibrium under these particular conditions." (Gesell, Krueger, Gorham and Bernthal, 1930a.)

It is conceivable that an accumulation of carbonic acid in the tissues might act to increase the lactic acid in the blood not necessarily as a result of an alteration in the rate of its formation or of its removal but because of a shift from the tissues to the blood. Such a shift has been proposed as a possible explanation for an increase in blood lactic acid under other experimental conditions (Gesell, Krueger, Gorham and Bernthal, 1930).

The accumulation of lactic acid in the presence of an abundance of oxygen suggests that oxidations are regulated by some factor other than the presence of oxidizable materials and sufficient oxygen. Might this factor be of the nature of an enzyme? If so we might explain the decreased oxidations (Bean, 1931) and consequent lactic acid accumulation during exposure to high oxygen pressure as being due to a poisoning of this enzyme. Recovery from the increased blood lactic acid on decompression might indicate a return of enzymatic function or a production of fresh unpoisoned enzyme. In support of this hypothesis we have during the recovery period a reduction in blood lactic acid and, as previously reported (Bean, 1931), a temporary overshooting of oxygen absorption. With a return to normal enzymatic control we would expect such increased oxidations in the presence of an abundance of oxygen and oxidizable materials. Failure of recovery might indicate an irreversible alteration in the enzymes or a permanent damage to cells involved in the production of enzymes. Perhaps a similar explanation for the effects produced by intravenous injection of sodium cyanide might not be amiss.

SUMMARY

The purpose of these experiments was to determine alterations in the content of blood lactic acid which might occur during exposure of animals to oxygen at pressures of about five atmospheres.

The experimental animals were dogs anesthetized with morphine and urethane. Periods of exposure to the increased pressure were usually of about thirty minutes' duration and were preceded and followed by administration of oxygen at one atmosphere. Decompression was carried out in stages, and over such periods of time as to avoid effervescence which might occur with too rapid decompression.

The preliminary exposure to oxygen at one atmosphere was frequently attended by a slight drop in the lactic acid content of the blood.

Subsequent breathing of oxygen at pressures of about five atmospheres usually was attended by an increase in blood lactic acid. Fifteen of nineteen experiments showed an increase in blood lactic acid while four showed no appreciable change. Recovery from the lactic acid increase was practically complete in six experiments on decompression. Six others showed only partial recovery and in three more the lactic acid continued to increase during and subsequent to decompression.

Respiratory minute volume was increased during exposure to high oxygen pressure in all experiments except one.

Evidence was presented to show that the lactic acid increase was not due to increased activity of the respiratory muscles.

Cellular damage resulting from high oxygen pressure was considered as a possible factor contributing to the lactic acid increase in the blood.

Intracellular acidity arising from a broken coördination of the dual function of the hemoglobin was discussed as a possible indirect cause for the lactic acid increase. The increased intracellular acidity might give rise to an augmented production of lactic acid by lowering of oxidations. It might also cause a shift in lactic acid from the interior of the cell to the blood.

The possibility of an enzymatic control of lactic acid removal was suggested. High oxygen pressure might have a toxic effect on the enzymes involved thereby accounting for the accumulation of lactic acid during exposure of the animal to high oxygen pressure.

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STUDIES ON THE KINETICS OF LACTATE FORMATION IN MUSCLE UNDER THE INFLUENCE OF IODOACETIC ACID

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Since the original observation by Lundsgaard (1930) that iodoacetic acid prevents the accumulation of lactate in striated muscle with only partial depression of excitability and contractility, considerable interest has been directed toward the possible mode of action of the substance. Dudley (1931) has found that iodoacetic acid inhibits the normal conversion of phenyl-glyoxal to mandelic acid by glyoxalase. Lundsgaard (1930) observed an accumulation of methyl-glyoxal in muscle following iodoacetic acid poisoning, and Case and Cook (1931), by means of fixation by sodium sulfite, have detected an accumulation of both methyl-glyoxal and pyruvic acid in normal muscle. Lundsgaard (1930) observed that glycogen breakdown progresses normally in iodoacetic acid treated muscle, but that hexose-diphosphoric acid ester accumulates. Although the mass of evidence seemed to point conclusively toward an inhibition of carbohydrate breakdown prior to the lactic acid stage, it occurred to the authors that one possible mode of alteration of the carbohydrate cycle, not previously investigated, had not been entirely ruled out by previously recorded observations.

The fact that hexose-diphosphoric acid ester accumulates in iodoacetic acid treated muscle in amount sufficient to have suggested to Lundsgaard (1930) the possibility of a stabilization of that substance, presented the possibility that iodoacetic acid might effect the removal of the lactic acid formed during the contraction by accelerating its conversion to hexose-diphosphoric acid ester in the early post-contraction period. During the investigation of this problem a number of interesting facts have come to light. These have to do particularly with the kinetics of lactic acid production in the presence of iodoacetic acid.

All intact muscles were frozen by immersion in liquid air before preparation of the protein-free filtrate. Precipitation of proteins was accomplished by the use of tungstic acid, by a procedure previously described (Visscher and Smith, 1930). Lactic acid was determined by the method of Friedemann, Cotonio, and Shaffer (1927).

Minced muscle was prepared by thorough grinding with sand in a mortar. Monoiodoacetic acid, Eastman, was used, unneutralized, throughout, since the concentra-

tion was lower in most cases than that used by other workers, and too low to affect the pH significantly. Merck lactic acid, 85 per cent, was diluted for use in those experiments requiring addition of lactic acid. Stimulation was uniformly done directly, in oxygen-free Ringer by means of condensor discharge usually spaced to allow an interval of 1 second.

1. *The fate of preformed or added lactic acid in iodoacetic-acid treated muscle.* If the effect of iodoacetic acid were an acceleration of lactate removal, it seemed reasonable to us to assume that lactic acid would be taken up from a lactic-acid bearing solution and converted into a hexose-phosphoric acid ester by iodoacetic acid treated muscle.

TABLE 1

Influence of iodoacetic acid upon added lactic acid

EXPERIMENT	IODOACETIC ACID ADDED	LACTIC ACID ADDED	LACTIC ACID BY ANALYSIS		TIME IN SOLUTION	TOTAL LACTIC ACID BY ANALYSIS—MUSCLE PLUS SOLUTION
			Muscle	Solution		
		per cent	*per cent*	*per cent*		*mgm.*
1 A (gastroc.)	1/20,000	0.039	0.045	0.038	4:00– 5:20	2.213
1 B (gastroc.)	0	0.039	0.049	0.036		2.192
2 A (sart.)	1/30,000	0.039	0.041	0.036	4:45– 5:45	2.040
2 B (sart.)	0	0.039	0.061	0.040		2.315
3 A (gastroc.)	1/10,000	0.039	0.040	0.038	5:10– 7:50	2.435
3 B (gastroc.)	0	0.039	0.042	0.039		2.462
7 A (sart.)	1/30,000	0.039	0.049	0.039	10:02–11:15	2.205
7 B (sart.)	0	0.039	0.057	0.036		2.133
8 A (gastroc.)	1/10,000	0.039	0.042	0.037	10:15–12:00	2.870
8 B (gastroc.)	0	0.039	0.043	0.036		2.855

Consequently, pairs of normal sartorius and gastrocnemius muscles from small winter frogs were placed in Ringer solution containing 0.039 per cent of lactic acid, and in the case of one muscle from each pair from 0.01 per cent to 0.0033 per cent of iodoacetic acid. Both muscles of a pair and the solutions in which they had been immersed were prepared for analysis after intervals varying from 60 to 160 minutes (table 1).

The findings do not indicate any significant variation between the total lactic acid content of the iodoacetic acid containing system and that of the iodoacetic-acid-free system. The lactic acid content of any individual muscle and the Ringer in which it had been immersed indicates an approximate diffusion equilibrium if account be taken of the inescapable water loss from the muscle to the slightly hypertonic immersion fluid. The use of

commercial, racemic lactic acid might be questioned, except for the fact that in all cases that lactic acid content of the iodoacetic acid containing system should have been the lower had the not insignificant amount of preformed sarco-lactic acid (the "resting" content) been resynthesized or otherwise removed. In a single experiment, an attempt to test the latter point, both muscles of a similar pair were fatigued, one placed in iodoacetic acid Ringer and the other in plain Ringer. At the end of a two-hour period the lactic acid content of each was essentially the same.

It occurred to us that a more crucial test of the problem might consist of the use of muscle poisoned by injection of iodoacetic acid into the intact animal. To this end iodoacetic acid in concentration of 0.165 mgm. per gram, based on the gross weight of the frog, was injected into the ventral

TABLE 2

Influence of iodoacetic acid upon the final concentration of lactate in macerated poisoned muscle

EXPERIMENT	TYPE AND WEIGHT OF MUSCLE	INCUBATION PERIOD	LACTIC ACID ADDED	LACTIC ACID FOUND	IODOACETIC INJECTED
		hour	*mgm.*	*mgm.*	
22 1	Thigh, 2.96 gram	2	10	9.42	1/6,000
22 2	Thigh, 2.80 gram	0	10	9.78	
23 1	Thigh, 3.51 gram	0	10	9.25	1/6,000
23 2	Thigh, 3.43 gram	2	10	10.10	

Calculated amount present if lactate accumulation had proceeded unchecked, on the basis of 0.5 per cent as an average final lactate concentration.

22, 1 ..... 24.80 mgm.
23, 2 ..... 27.15 mgm.

lymph sac, and thigh muscle taken after 40 minutes. Muscle from one thigh of a single animal was frozen immediately and its proteins precipitated after the addition to the system of 10 mgm. of lactic acid in 10 cc. of Ringer. The corresponding muscles of the opposite thigh were ground with sand in a mortar, 10 mgm. of lactic acid in 10 cc. of Ringer added, and the mixture subjected to two hours' incubation at room temperature. Within the limits of the error introduced through the difficulty of quantitative transfer from the mortar while holding the total volume low, the final total lactate concentration is essentially the same in the two cases. Two typical experiments are shown in table 2. It is noticeable that no new lactate accumulation took place in the muscle itself after this treatment. The calculation appended to table 2 shows this in a striking manner.

2. *The effect of concentration of iodoacetic acid on irritability, and upon lactate accumulation in intact and minced muscle.* Since we had obtained

apparently complete inhibition of lactate production by the comparatively low concentration of 0.165 mgm. per gram, it seemed important to us to make an attempt to determine the liminal effective concentration of iodoacetic acid.

TABLE 3

Influence of concentration of iodoacetic acid upon lactate production

EXPERIMENT	IODOACETIC INJECTED	TIME AFTER INJECTION	PROCEDURE	LACTIC ACID FOUND
		minutes		*per cent*
27 A	1/5,000	37	Resting	0.019
27 B			Fatigued	0.013
22 A	1/6,000	40	Fatigued	0.020
22 B			Fatigued	0.023
29 A	1/15,000	35	Resting	0.015
29 B			Fatigued	0.019
30 A	1/15,000	45	Resting	0.017
30 B			Fatigued	0.021
32 A	1/15,000	60	Resting	0.029
32 B			Fatigued	0.031
33 A	1/15,000	60	Resting	0.016
33 B			Fatigued	0.029
34 A	1/20,000	30	Resting	0.028
34 B			Fatigued	0.033
35 A	1/20,000	30	Resting	0.032
35 B			Fatigued	0.028
31 A	1/30,000	30	Resting	0.091
31 B			Fatigued	0.195
		hours		
9 A	1/30,000	2	Unstimulated	0.035
9 B	1/30,000	2	Stimulated	0.122
10 A	1/30,000	2¼	Unstimulated	0.022
10 B	1/30,000	2¼	Stimulated	0.119

To this end frogs were injected with concentrations of iodoacetic acid varying from 0.2 mgm. per gram to 0.033 mgm. per gram, and dissection of the gastrocnemius muscles made following intervals of from 30 to 135 minutes. One muscle of each pair was frozen immediately as a control, its mate stimulated to fatigue and frozen subsequently. Concentrations of

iodoacetic acid of 0.05 mgm. per gram and above uniformly allowed no lactate accumulation, while 0.033 mgm. per gram showed incomplete inhibition of lactate production (table 3).

Tissue irritability did not appear to correspond as closely to the concentration of iodoacetic acid as did the inhibition of lactate production, muscles from animals injected with 0.033 mgm. per gram occasionally showing complete depression of irritability, and muscles from animals receiving high concentrations frequently responding to stimuli. Lactate accumulation was quite constantly inhibited, however, in concentrations above 0.033 mgm. per gram. The depression of irritability raised a further significant question. Could not the depression of irritability and the feeble response to stimuli be responsible for the low lactate accumulation in poisoned muscles? A further possibility also presented itself. Might

TABLE 4

Lactate accumulation in chloroform rigor

EXPERIMENT	IODOACETIC INJECTED	INTERVAL BETWEEN INJECTION AND DISSECTION	LACTIC ACID FOUND	
			per cent	
23 A	1/6,000	10:45–11:45	0.013	Muscle not irritable
23 B	1/6,000	10:45–11:45	0.014	Chloroform rigor
28 A	1/5,000	3:08– 3:50	0.019	Muscle not irritable
28 B	1/5,000	3:08– 3:50	0.034	Chloroform rigor
Control			0.020	Resting
Control			0.509	Chloroform rigor
Control			0.490	Chloroform rigor

not the reconversion of lactic acid to hexose phosphate, if such occurred, proceed at such a rate that our unavoidable delay in freezing and precipitating made it impossible for us to determine the lactic acid actually formed? Subjecting muscle to treatment such as chloroform rigor, with the consequent rapid great increase in lactic acid, independent of natural irritability, should offer an opportunity to test both of these questions. Freshly dissected gastrocnemius muscles from poisoned frogs were subjected to the action of chloroform and analyzed in the usual manner. Lactate accumulation did not occur with such treatment as is evident from table 4, in which is also indicated the high lactate accumulation normally accompanying this procedure. It seems conclusive then that iodoacetic acid in high concentrations inhibits lactate accumulation, and does so quite independently of natural irritability. Furthermore, it becomes more probable that delay incident to the manipulation of freezing in liquid air is not a factor in masking lactate formation by allowing its removal.

It seemed advisable, however, to study the effect of concentration in another, quite different, type of experiment, namely, in minced muscle. Frogs were injected as before with varying concentrations of iodoacetic acid, from 0.2 mgm. per gram to 0.033 mgm. per gram. Thigh muscle from an individual animal was grossly minced with scissors and shuffled thoroughly, and in most cases divided into three portions. One large portion was weighed, ground with a few cubic centimeters of Ringer in a mortar without having been previously frozen, and set aside for incubation at room temperature. A second protion was frozen and prepared for the lactate determination immediately. The third portion was ground without freezing and then precipitated with tungstic acid, in order to induce the maximum lactic acid production possible through extensive injury (table 5).

TABLE 5

The effect of concentration of iodoacetic acid upon lactate accumulation in macerated muscle

EXPERIMENT	TYPE AND WEIGHT OF MUSCLE	IODOACETIC INJECTED	INCUBATION PERIOD	LACTIC ACID POUND	
			hours	*per cent*	
28 1	Thigh, 3.61 gram	1/5,000	3	0.008	
28 2	Thigh, 3.67 gram		0	0.008	
19 1	Thigh, 1.78 gram	1/6,000	0	0.011	Macerated before freezing
19 2	Thigh, 1.66 gram		0	0.011	Frozen, then macerated
19 3	Thigh, 3.28 gram		2	0.014	
30 1	Thigh, 1.76 gram	1/15,000	3	0.015	
30 2	Thigh, 2.03 gram		0	0.012	Macerated before freezing
31 1	Thigh, 3.07 gram	1/30,000	3	0.230	
31 2	Thigh, 2.49 gram		0	0.128	Macerated before freezing
20 1	Thigh, 2.01 gram	Control	0	0.126	Macerated before freezing
20 2	Thigh, 2.52 gram		0	0.081	Frozen, then macerated
20 3	Thigh, 4.37 gram		2	0.460	

High concentrations of iodoacetic acid inhibited lactate accumulation completely in all portions, even when minced prior to freezing, in great contrast to the normal muscle. Normal muscle taken as a control showed a high original level, as would be expected due to injury in dissection, a still higher level following grinding prior to freezing, and the usual high lactate accumulation following incubation. Thirty-three thousandths milligram of iodoacetic acid per gram of tissue only partially inhibited lactate accumulation, in complete accord with the results obtained in intact muscle. It is obvious that iodoacetic acid affects the production of lactic acid in minced muscle.

3. Effect of iodoacetic acid on the rate of lactic acid production in intact muscle. It seemed apparent to us that the final concentration of lactate in iodoacetic acid poisoned muscle following any experimental treatment depends upon the concentration of iodoacetic acid to which the muscle has been subjected. This fact suggested the fundamental importance of turning toward an investigation of the manner in which the carbohydrate-breakdown cycle is modified.

Evidence presented by the majority of the foregoing experiments pointed toward the conclusion that iodoacetic acid does not alter the rate of disappearance of lactic acid. Given equal numbers of stimuli and constant time intervals between beginning of stimulation and the immersion in liquid air, there occurs in normal muscle a high final lactic acid content, in lightly poisoned muscle a lower final content, and in heavily poisoned muscle a complete inhibition of lactic acid production. It is a matter of common observation that a delayed lactic acid production in normal muscle is difficult to detect by chemical means, regardless of the speed with which the control muscle is frozen following stimulation. This partial inhibition of lactic acid formation encountered at low concentrations therefore raised a further question. Does iodoacetic acid effect a limitation of the final concentration to which lactic acid may accumulate? This seemed unlikely. It seemed more probable that the rate of the reaction of which lactic acid is the end product is so retarded that at equal intervals of time following any disturbance which tends to shift the precursor-lactic acid equilibrium to the right, the reaction in poisoned muscle and in normal muscle can not have proceeded to the same extent.

Iodoacetic acid was injected in the usual manner, in two concentrations, 0.165 mgm. per gram, and 0.033 mgm. per gram, and dissection of the gastrocnemius muscles made after intervals of from 35 to 70 minutes. One muscle of each pair was fatigued and frozen immediately, the other was fatigued and placed for 20 minutes in a paraffin chamber through which nitrogen flowed constantly. Experiments 15 and 16, table 6, show a considerable lactate accumulation during the post-contraction interval, while normal muscles and muscles poisoned with higher concentration of the drug show no accumulation. The delayed lactate production, which may be termed the "recovery" lactate production in view of recent observations concerning the order of chemical events in the contraction process (Lippman and Meyerhof, 1930) and the function of lactic acid formation (Lundsgaard, 1930) indicates that the fundamental alteration in iodoacetic acid poisoned muscle is an inhibition of the rate of lactic acid formation. That this rate may become infinitely slow as the iodoacetic acid concentration increases is indicated by the complete suppression of lactate accumulation encountered above 0.05 mgm. of iodoacetic acid per gram.

4. The influence of concentration of iodoacetic acid upon the rate of lactic

acid production in minced muscle. The foregoing observation that the final lactate content in both minced and intact muscle is dependent upon the iodoacetic acid concentration and the conclusion that this is the resultant effect of an inhibition of the rate of lactic acid formation, led us to an attempt to study the uncomplicated effect of concentration of iodoacetic acid upon the rate of lactic acid formation.

Large bull frogs, R. catesbiana, were killed by transecting the spinal column anterior to the terminal lumbar vertebra, followed by a transverse

TABLE 6

Influence of iodoacetic acid upon rate of lactate production

EXPERIMENT	IODOACETIC INJECTED	TIME AFTER INJECTION	PROCEDURE	LACTIC ACID FOUND	
		minutes		*per cent*	
15 A	1/30,000	35	Fatigued	0.054	Frozen immediately
15 B			Fatigued	0.128	In N_2 20 minutes after stimulation
16 A	1/30,000	70	Fatigued	0.031	Frozen immediately
16 B			Fatigued	0.123	In N_2 20 minutes after stimulation
17 A	1/6,000	40	Fatigued	0.018	Frozen immediately
17 B			Fatigued	0.018	In N_2 20 minutes
18 A	1/6,000	55	Fatigued	0.015	Frozen immediately
18 B			Fatigued	0.015	Frozen immediately
19 A	1/6,000	60	Non-irritable	0.012	Frozen immediately
19 B			Non-irritable	0.013	Frozen immediately
20 A	Control		Fatigued	0.242	Frozen immediately
20 B			Fatigued	0.256	In N_2 20 minutes
21 A	Control		Fatigued	0.210	Frozen immediately
21 B			Fatigued	0.215	In N_2 20 minutes

cut across the head at the angle of the mouth, and destruction of the spinal cord with a probe posteriorly from the latter cut. As much as possible of the total muscle of the hind legs was removed and frozen by immersion in liquid air after division into suitably small pieces with scissors. The frozen pieces were forced through a meat grinder, previously cooled with liquid air, and the resulting coarse particles ground with a pestle to a fine powder in a pre-cooled mortar. The powder was thoroughly mixed, covered to prevent condensation of water vapor upon it, and kept frozen by repeated applications of liquid air. A separate set of beakers was pre-

pared for each concentration of iodoacetic acid to be used, each beaker in the set corresponding to a definite incubation time. Into each beaker were placed 10 cc. of Ringer containing such an amount of iodoacetic acid that its final concentration following the addition of a pre-determined weight of muscle powder would be that desired. The beakers were then weighed, and covered to prevent evaporation. A set of beakers containing Ringer alone served as a control. Muscle powder was first roughly apportioned to each beaker by means of a cooled spatula which delivered the desired weight of tissue within satisfactory limits when uniformly filled. The beakers were weighed on a quick weighing quadrant balance, sensitive to

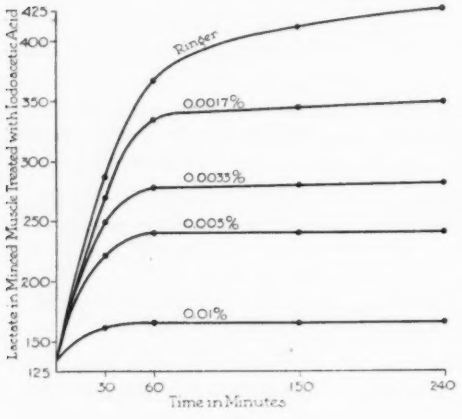

Fig. 1

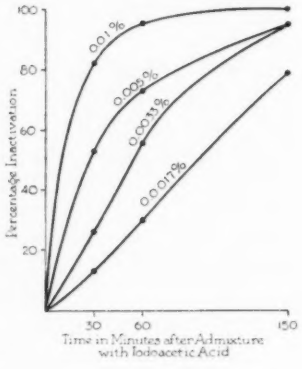

Fig. 2

Fig. 1. Lactic acid formation in minced muscle treated with the concentrations of iodoacetic acid noted on the separate curves in comparison with the rate of formation in Ringer's fluid.

Fig. 2. The rate of inactivation of the enzyme system by iodoacetic acid. The percentage inactivation of the enzyme system is determined over the course of time for several concentrations of iodoacetic acid.

2.5 mgm., to determine the exact weight of muscle used. The tissue mass was then dispersed with a stirring rod. One beaker from each set was taken at zero time, the proteins being precipitated as described above as quickly as possible after weighing. Similarly, one beaker from each set was taken at 30, 60, 150 and 240 minutes.

Except for the expected variations in the original lactate content, the results of 6 complete experiments agree closely. A composite graph of a typical experiment is shown in figure 1.

There was a uniformly approximately complete inhibition of lactic acid production by 0.01 per cent iodoacetic acid, and a graded inhibition by

lower concentrations, proceeding in a strikingly regular fashion. One point of similarity between all the experiments has led to a more critical examination of our results from a physico-chemical point of view.

It is to be noted that lactic acid formation in muscle powder in the presence of iodoacetic acid tends toward a maximum concentration peculiar to the particular percentage of iodoacetic acid. In no case does the final concentration of lactic acid in the presence of iodoacetic acid become equal to that in the presence of plain Ringer, even at the low concentration of 0.0017 per cent, over a period of 4 hours. Between 60 and 150 minutes, and between 150 and 240 appreciable lactic acid continues to be formed in muscle powder in Ringer, while the slope of the curves representing lactic acid formation in the presence of iodoacetic acid has uniformly approached zero.

If iodoacetic acid inhibits the action of glyoxalase, it appears to us that this inhibition must be effected in one of two ways. Iodoacetic acid might act as a retarding agent without itself entering into any chemical reaction with any component of the system, in the manner of a negative catalyst. This possibility is rendered unlikely by the observation that the inhibition of lactic acid formation in the case of all concentrations of iodoacetic acid progressively increases with time. It seems reasonable that if any lactic acid production is to be noted in the early minutes, it should proceed at that retarded rate, eventually to reach a concentration equal to that in the uninhibited system at infinite time. Our observations obviously do not fit in with this concept. If, on the other hand, the inhibition is effected by an inactivation of some component of the system, the observations recorded above are those which might be expected. If the action of iodoacetic acid were an inactivation of glyoxalase, this inactivation proceeding at a rate determined by the concentration of one of the reactants, in this case iodoacetic acid, then a progressively increasing inhibition of lactic acid formation would be expected as a result.

That a progressive increase in inhibition does occur is shown by figure 2, where the rate of inactivation of the enzyme system is calculated and the average from three experiments is plotted against time. During the later intervals there is also a decrease in the rate of lactic acid formation in the Ringer control, as the lactic acid maximum is approached. The apparent reason for this progressive decrease in rate in Ringer is the gradual depletion of the substrate. Since it is a matter of common experience that lactic acid accumulation in muscle brei proceeds to depletion of carbohydrate stores only after the addition of phosphate, it seems to be an essential part of the substrate in such a system, and its concentration may in this instance be the limiting factor. It is obvious that a direct comparison of the increase of lactic acid in the iodoacetic acid Ringer and in the plain Ringer for corresponding time intervals is open to serious criticism. At

the beginning of any time interval except the first, since lactic acid production has not proceeded to the same extent in both Ringer and iodoacetic acid Ringer, the concentration of substrate likewise cannot be identical, and the true inhibition by iodoacetic acid is partially masked by the "substrate depletion" inhibition of the control system.

In order to calculate the inhibition of the enzyme system due to iodoacetic acid, it is necessary to measure the extent of inhibition not at identical times in the poisoned and the unpoisoned system, but at identical concentrations of the substrate reactants. Thus in figure 1, for example, in determining the percentage inhibition of the system with 0.0033 per cent iodoacetic acid at 60 minutes' time, one cannot compare the rate during the next hour with the rate of the control experiment during that same hour, because in the latter nearly twice as much of the substrate had already been used up at that point. It is therefore necessary to compare the slope of the lactic acid formation curve in the iodoacetic treated muscle at 60 minutes with the slope of the curve for the untreated muscle at a corresponding concentration of substrate reactants, which happens to have existed in this experiment at approximately 28 minutes after the onset of the reaction. This calculation depends upon the assumption that the lactic acid formed is a reliable measure of the amount of original reactant used up. This assumption is undoubtedly permissible unless there is an important intermediary product between the reaction influenced by iodoacetic acid and the lactic acid. This seems extremely unlikely, because iodoacetic acid is known to inhibit glyoxalase which is itself the enzyme responsible for the last step in the formation of lactic acid.

The rate of lactic acid formation in the iodoacetic system is expressed as the per cent of the rate under similar conditions in the normal system. This figure is subtracted from 100 in order to express the results as percentage inactivation. It is apparent from figure 2 that the rate of inactivation is a function of the concentration of iodoacetic acid and that it tends towards complete inactivation at all concentrations, although in the lowest concentrations used the inactivation is not rapid enough to be completed before a large share of the substrate has been acted upon by the active enzyme system.

If one plots the logarithm of the enzyme concentration against time during the inactivation by iodoacetic acid, one finds a linear relation between the two which must be interpreted as indicating that in the kinetics of the process at a given concentration of iodoacetic acid the concentration of the enzyme itself limits the rate of its inactivation. Thus it appears to be a reaction of the first order, although obviously it is not monomolecular. It seems likely that the iodoacetic acid itself does not enter into permanent combination with the enzyme.

Lohmann (1931) in a study on hexose-phosphoric acid esterification in

muscle extract has called attention to the fact that the action of mono-iodoacetic acid differs from that of fluoride. Whereas poisoning by the latter is practically instantaneous, poisoning by mono-iodoacetic acid is a "time reaction," requiring as long an interval as 60 minutes for maximum ester accumulation. He postulates, in the case of mono-iodoacetic acid, an irreversible alteration of the protein component of the enzyme system. Meyerhof and Boyland (1931) have also established that poisoning of frog sartorii by immersion in mono-iodoacetic acid is a process involving considerable time, which they interpret as being determined by the rate of diffusion into the interior of the muscle. It seems that in our experiments with powdered muscle diffusion rate does not play a rôle after the first few minutes.

CONCLUSIONS

1. Preformed or added lactic acid does not disappear during anaerobic recovery in iodoacetic acid poisoned muscles.

2. Muscles poisoned with low concentrations of iodoacetic acid show only partial inhibition of lactic acid production as a result of stimulation. Iodoacetic acid depresses the irritability of muscles but the inhibition of lactate formation is not due to this non-irritability, since chloroform rigor fails to produce lactic acid in poisoned muscles.

3. With low concentrations of iodoacetic acid there is in contrast to the situation in normal muscles and in those treated with high concentrations of iodoacetic a large delayed lactic acid production following stimulation.

4. The rate of lactate formation in minced muscle is inhibited by iodoacetic acid in direct proportion to the concentration of the poison. The inhibition increases progressively, indicating an inactivation proceeding with measurable velocity.

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THE RESPIRATORY METABOLISM OF PANCREATIC DIABETES IN CATS

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In pancreatic diabetes, Falta, Grote and Staehelin (1907) showed that the dog's metabolism was increased by 42 per cent. Murlin and Kramer (1913) obtained a similar result with their dog. Enderlen, Glatzel and Pú (1928) found an increase above the basal metabolism ranging between 3 and 28 per cent. Hédon (1927) in his pancreatectomies left a remnant of the tail just under the skin to supply the pancreatic hormone. When the animal had fully recovered from the laparotomy, then the graft was removed and the metabolism measured. Eight out of eleven dogs showed an increased metabolism. One diabetic animal to which insulin was given showed a normal metabolism which increased when the insulin was withdrawn.

In human diabetes Benedict and Joslin (1912) found an increase of about 20 per cent. Allen and DuBois (1916) concluded from their own results and a recalculation of those obtained by Magnus-Levy, Mohr, and Benedict and Joslin, that although some results were high the average metabolism of diabetes showed no marked increase. Joslin (1923) states concerning his work that "23 per cent of the total number of observations, representing 27 per cent of the patients for whom the Harris-Benedict standard was used in the post-absorptive state, varied more than 20 per cent above or below the center of the normal zone of metabolism."

It seems surprising that the dog should always show an increased metabolism in diabetes and the results on man be so variable. Perhaps the explanation lies in the fact that in human diabetes the islet cells are almost never entirely destroyed and therefore the disease is not as complete as after extirpation. At least, under the circumstances, it appeared worth while to study the metabolism of diabetes in another animal. This was done in cats.

METHOD. The open-circuit method of measuring metabolism was used. Compressed air under a constant pressure was passed through the animal chamber at a rate such that the outgoing air contained about 1 per cent CO_2. The animals were kept comfortable by having the lower half of the respiration chamber in a water bath at 30°C. and by warming the ingoing

air to the same temperature. Taking these precautions, we found that our cats would become quiet very quickly and would often remain so for hours. A graphic record of activity told us when the animal was still, and at half-hour intervals thereafter we sampled the outgoing air and later analyzed it for carbon dioxide and oxygen. Usually two out of three metabolism measurements by this method checked satisfactorily for our purposes.

The analyses of the samples were made in three gas analyzers of the 10 cc. Carpenter type. Results on the same sample in different analyzers were checked within 0.04 per cent.

No estimations of urinary nitrogen were made. The metabolism calculations are based on carbohydrate and fat combustion alone, which of course makes all the figures too high. It is possible that the error involved in not measuring urinary nitrogen is more serious in the diabetic than in the normal animals. Yet if we assume that these cats burn only protein and that 58 per cent of this is converted to sugar and excreted, our calculations would not have to be lowered by more than 13 per cent. This would still leave a marked difference between the metabolism of the normal and the diabetic cat.

The Meeh formula as given by Lusk (1928) was used in the estimations of surface area. The results are given in calories per square meter per hour.

In most of our cats, the metabolism was measured a few days prior to pancreatectomy. After the removal of the pancreas, urine sugar and acetone were qualitatively determined by Benedict's and Rothera's tests respectively. Usually about four units of insulin were given on the second day after the operation and this amount was increased until the urine became acetone-free. It was impossible for us to regulate the dosage of insulin to make the urine free from sugar without at the same time producing hypoglycemia and convulsions.

Results. Cats differ greatly from dogs in their responses to diabetes. For example, Soskin (1930) has had a diabetic dog on a protein diet that survived thirty-three days after the withdrawal of insulin. In this animal the acetone in the urine gradually rose to a maximum on the fourth day and then diminished. In some dogs the acetone actually disappeared from the urine.

Cats on a diet of salmon, pancreas and milk usually show acetone in the urine by the second day after stopping insulin and may even die then, though they usually survive until the fourth day when the acidosis becomes very severe. At this time insulin treatment is almost always ineffective in bringing about recovery. Acidosis is less likely to appear if a high carbohydrate diet is given but the survival period is not changed.

The metabolism of our cats was determined before the operation and

then at least a week after recovery. The results of 41 observations on 19 normal cats showed an average metabolism of 29.3 ±0.4 calories per square

TABLE 1

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			kgm.		
514	Dec. 16		2.8	0.85	30.0
					30.7
	Jan. 4	Depancreatized			
	Jan. 13	2nd day without insulin	2.0	0.70	50.6
				0.67	48.3
				0.67	48.0
	Jan. 14	3rd day without insulin	2.0	0.69	39.2
				0.73	39.0
	Jan. 20	With insulin	2.0	0.75	40.5
	Jan. 29	With insulin	1.6	0.67	43.3
				0.71	42.5
516	Dec. 17		3.7	0.78	32.8
				0.71?	32.1
	Dec. 21	(Partial thyroidectomy)			
	Dec. 29		3.1	0.78	31.6
				0.77	30.7
	Jan. 4	Depancreatized			
	Jan. 11	With insulin	2.8	0.75	41.8
				0.75	41.8
	Jan. 14	With insulin	2.7	0.65	40.1
				0.70	40.2
	Jan. 21	With insulin	2.6	0.76	40.1
				0.70	39.4
991	Sept. 18			0.82	31.5
	Sept. 22		2.7	0.85	31.6
	Sept. 23	Depancreatized			
	Oct. 1	Insulin 24 hours before	2.2	0.74	39.0
				0.71	39.8
	Oct. 3	Insulin 24 hours before	2.1	0.76	39.9
				0.77	38.4
	Oct. 7	2nd day without insulin	2.0	0.68	42.2
				0.71	38.5
	Oct. 14	Insulin 24 hours before	1.8	0.81	43.3
				0.83	43.2

meter per hour, the maximum single observation being 34.8 calories. The respiratory quotients for the series averaged 0.75 ±0.006.

With one exception, these cats, made diabetic, showed a high metabolism on the second day without insulin. The mean of 28 observations on 10 cats

that survived for two weeks or more is 41.7 ±0.4, the lowest result being 36.6 calories. The respiratory quotients averaged 0.70 ±0.004. The increase for the series was 42 per cent. The smallest rise for any animal was 13 per cent. The constancy of the increased metabolism is very surprising in view of the marked inanition shown by some (see table 1). This is in contrast to the results obtained by Allen and DuBois (1916) and Joslin (1923) on human diabetic subjects undergoing the Allen treatment in which under-nutrition was common and the average metabolism fell below normal.

The one exception which we have not included in the series above was No. 129 shown in table 2. This cat had a 13 per cent increase in metabolism on the second day without insulin, but its heat production in diabetes was

TABLE 2

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			kgm.		
129	Jan. 5	Depancreatized			
	Jan. 9	With insulin	2.3	0.71	29.2
				0.72	29.3
				0.71	29.7
	Jan. 11	2nd day without insulin	2.2	0.72	31.0
				0.66	34.9
				0.65	33.5
	Jan. 12	3rd day without insulin	2.1	0.70	34.8
				0.71	33.1
	Jan. 13	4th day without insulin	2.0	0.69	47.9
				0.68	48.5
				0.73	46.2

so close to the results for normal cats that it seemed possible that the pancreas had not been completely removed. Yet, on the fourth day without insulin, the metabolism jumped to a very high level and the animal died in severe acidosis in spite of receiving insulin.

We expected that all diabetic cats would show a normal metabolism when given insulin. This, however, was not true. Varying the dosage of insulin or the time interval between the injection and the metabolism measurement failed in a number of cats to bring the heat production down to normal.

A few animals, for unknown reasons, did show normal metabolism when given insulin after pancreatectomy. The results on these animals are given in table 3. It will be noted that the second day after the withdrawal of insulin the metabolism had risen to the diabetic level.

The removal of the head of the pancreas, leaving the tail to supply

insulin, brought about no change in metabolism. In 25 observations on such cats, the respiratory quotients averaged 0.75 ±0.001 and the

TABLE 3

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			kgm.		
191	Sept. 22		1.9	0.80	27.3
	Sept. 25	Depancreatized			
	Oct. 6	Given insulin	?	0.76	29.3
	Oct. 8	Given insulin and glucose	1.9	0.93	30.3
	Oct. 13	2nd day without insulin	1.7	0.70	41.5
	Oct. 15	2nd day without insulin	1.5	0.69	41.0
521	Dec. 22	Head of pancreas removed			
	Jan. 7		2.5	0.80	29.1
					30.7
	Jan. 12	Rest of pancreas removed			
	Jan. 20	2nd day without insulin	2.3	0.74	39.4
				0.69	42.7
				0.68	43.9
	Jan. 22	With insulin	2.3	0.72	
				0.69	31.0
				0.68	32.4
532	Jan. 18	Depancreatized			
	Jan. 26	1st day without insulin	2.9	0.71	30.3
				0.74	29.1
	Jan. 27	2nd day without insulin	2.8	0.76	38.6
				0.74	40.2
				0.74	38.5
534	Jan. 28	Depancreatized			
	Feb. 6	4 units insulin 24 hours before	2.3	0.68	39.5
				0.70	38.8
	Feb. 10	10 units insulin 24 hours before	2.1	0.74	32.5
	Feb. 13	No insulin for 48 hours	1.9	0.74	43.6
				0.71	46.6
992	Sept. 23		2.5	0.82	29.1
	Sept. 28	Depancreatized			
	Oct. 6	Insulin 24 hours before	2.2	0.74	29.1
				?	30.0
	Oct. 8	Insulin and glucose 24 hours before	1.9	0.70	42.4
				0.70	41.1

metabolism per square meter per hour was 29.4 ±0.6—the same as unoperated cats. Table 4 shows observations on such a cat.

We are now investigating the reason for the rise in metabolism after complete pancreatectomy.

TABLE 4

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			kgm.		
503	Nov. 16		2.35	0.83	31.0
			2.35	0.80	32.6
	Nov. 17			0.83	30.8
	Nov. 30	Partial pancreatectomy			
	Dec. 4		2.0	0.70	31.8
				0.75	28.1
				0.75	28.0
	Dec. 7		1.9	0.77	28.9
				0.80	32.5

CONCLUSIONS

1. Normal cats in post-absorptive condition have an average metabolism of 29.3 ±0.4 calories per square meter per hour and a respiratory quotient of 0.75 ±0.006 (see tables 1 and 3).

2. If the head of the pancreas is removed, cutting off the supply of pancreatic juice to the intestine, the metabolism remains unchanged. The averages for these animals are 0.75 ±0.001 for the respiratory quotient and 29.4 ±0.6 for the metabolism (see table 4).

3. Complete removal of the pancreas lowers the respiratory quotient to 0.70 ±0.004 and increases the metabolism more than 40 per cent to 41.7 ±0.4 calories (see tables 1, 2 and 3). This occurs even in under-nutrition.

This work was done at the suggestion of Dr. W. B. Cannon.

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THE PLACE OF THE RED NUCLEUS IN THE POSTURAL COMPLEX

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It has been believed in various quarters that the differences in behavior between decerebrate animals of the Sherrington type and those in which the transection has been made through or in front of the thalamus depends upon the structural and functional integrity of the red nucleus in the latter instances. The experiments of Rademaker (1926) have tended to support this assumption, the work of this investigator indicating that section of the decussation of Forel or destruction of the red nuclei by gross lesions induces a condition of greatly increased extensor tonus with loss of labyrinthine and "body on the body" righting reflexes, and renders the animal practically helpless. It has appeared not unlikely, however, that Rademaker's conclusions were based upon surgical procedures which may have involved other important centers in the brain stem, and it has been suggested that the radical effects produced were due to injury of certain portions of the vestibular complex as well as of the red nucleus. The position of the latter, deeply situated as it is, precludes the use of ordinary techniques in attempting its ablation. Lesions produced in the conventional manner are almost sure to damage so great a mass of tissue as to detract from the reliability of the results.

The present writers have recently reported a series of observations on cats in which the greater portions of the red nuclei were destroyed by the use of the Horsley-Clarke stereotaxic instrument (Ingram and Ranson, 1932). This apparatus, the use of which has been discussed in earlier publications (Ingram, Ranson, Hannett, Zeiss and Terwilliger, 1932; Ingram, Ranson and Hannett, 1932), enables one to place small electrolytic lesions in the interior of the brain with a minimum of damage to regions outside the structure which it is wished to destroy. Following the destruction of that portion of the red nucleus caudal to the middle or rostral fibers of the third nerve, it was found that the cats retained their ability to right themselves, stand and walk to a degree which enabled them to live normal lives. According to Rademaker, such lesions eliminate the labyrinthine and "body on the body" righting reflexes, leaving the optic, cervical and "body on the head" reflexes intact. If this is so, the two latter must have

been sufficient in our cases, since after bilateral enucleation of the eyes the animals were still able to get on their feet and walk. Increase in tonus of the extensor muscles was also observed in this group of cats. These tonus changes were of mild character, insufficient to interfere with locomotion, and were noticeable only under suitable conditions. There were also certain abnormalities of the gait, such as dysmetria, poor coördination, and a slight degree of ataxia.

In this series of experiments the greater portion of each red nucleus, or its descending connections, was destroyed, but in practically every instance a small part of the rostral, diffuse portion of the nucleus was left, rostral to the third nerve. Since, according to Mussen (1927), lesions in this latter region cause symptoms which are quite different from those following damage to the pars compacta, and since it was desirable to further check and extend previous observations, another group of operations was performed in attempting to eliminate the red nucleus in its entirety. The results of these efforts are reported here.

Methods. The Horsley-Clarke stereotaxic instrument makes possible the introduction of a bipolar needle electrode to any point within the brain whose location has been determined previously in terms of the orientation planes of the instrument. Passage of a direct current between the poles of the electrode produces, at this point, an electrolytic lesion the extent of which depends upon the amount and duration of the current used. The electrodes are made of nichrome wires cemented together and reinsulated with chlorinated rubber solution. The bare tips of the wires are less than one millimeter apart, making possible the confinement of the influences of the current to a restricted area. For total destruction of the red nucleus a row of small lesions, one millimeter apart, was produced in the latter over a total extent of five millimeters rostrocaudally. A current of about one milliampére and of thirty to sixty seconds' duration was used. Lesions so placed fuse together to form a single elongate lesion affecting the nucleus ruber or its connections without serious damage to other portions of the brain stem. In many instances the red nuclei were directly destroyed, in others the lesions were placed so that the rubro-spinal tracts or the decussation of Forel were interrupted, with the nuclei themselves but partially involved. In the cases to be described the rubro-spinal and rubro-reticular tracts were completely interrupted either at their sources in the red nucleus, in the decussation of Forel, or in the proximal part of their course after crossing.

These experiments were all of the so-called acute type; no aseptic precautions were taken and the animals were killed within twenty-four hours after operation. The lesions were produced under ether anesthesia. Observations of the post-operative behavior of the animals were made after sufficient time for recovery from the anesthetic had elapsed. The ability of each animal to right itself, stand and walk was carefully studied, as was

the state of tonus of the extensor muscles. Changes in the degree of tonus were readily observed when the animal was suspended in a sort of canvas hammock with the legs protruding through holes. Normal animals in this situation ordinarily hold the limbs flexed and offer no consistent resistance to passive flexion of the legs although variable voluntary resistance is often encountered, but if there is any extensor hypertonus the limbs are held more or less extended and moderately stiff. There is under such circumstances a certain amount of resistance to passive flexion induced by grasping the leg above the wrist or ankle and moving it forcibly back and forth. The rigidity is more regularly elicited when the upward pressure is exerted against the pads of the toes in the position for the positive stütz reflex. With practice, this procedure offers valuable means of estimating the degree of slight changes in extensor tonus.

At the conclusion of the experiment the animal was killed, and the brain fixed by injecting the head with a solution of formaldehyde, U.S.P., 1:10. The brain stem was removed, imbedded in celloidin, and cut into fifty micron sections, every fourth one of which was stained for myelinated fibers by Weil's method, or with cresyl violet for cells, and mounted. Outline drawings of certain of these sections were made with the aid of the Edinger drawing apparatus to facilitate the determination of the extent of the lesions and the structures involved.

Observations. The cases to be discussed include seven cats in which the red nuclei or their descending connections were completely destroyed. Five of these were able to right themselves, stand and walk, even when the visual righting reflexes had been eliminated by blindfolding or enucleation of the eyes. Of the other two, one could not stand or walk; the other could stand, could right itself to some extent, but did not walk. Those able to progress will be discussed first and a brief description of each animal's behavior given. The accompanying figures illustrate the extent of the lesion in one of these cases, cat 231, for which a more complete protocol will be included.

Cat 231 suffered elimination of the optic righting reflexes by enucleation. Following production of the lesions involving the red nuclei and recovery from the anesthetic it was able to right itself from either side when placed on the ground. It walked with a poorly coördinated gait, extending the forelimbs far forward, while the hindlimbs tended to shoot back or to the side. Frequently the feet were poorly placed, so that the weight was thrown upon the dorsum of the wrist or upon the subverted toes of the hind feet. There was a tendency to turn in a circle to the right. When suspended in the hammock this animal was able to flex all four limbs and hold them flexed voluntarily, but when at rest all except the left hindlimb were extended. There was a marked stütz reaction in all except the left hindlimb, in which it was moderate. Moderate resistance to passive flexion was observed in all but the left hindlimb, in which it was slight.

The extent of the lesions in this case is illustrated in figures 1 to 6. The first two figures show the rostral poles of the red nuclei to have been completely destroyed, the lesions also involving the rostral portions of the rubro-spinal tracts; the section shown in figure 2 was taken 0.7 mm. caudal to that in figure 1. Figure 3 was taken from a level approximately 0.5 mm. caudal to figure 2; figure 4, 0.65 mm. caudal to figure 3; figure 5, 0.65 mm. caudal to figure 4; and figure 6, 0.1 mm. caudal to figure 5. The last four figures show that while the medial portion of the left and lateral portion of the right red nucleus escaped destruction, the rubro-spinal tracts were completely destroyed. The lesions were confluent and the zone of injury continuous rostro-caudally. Structures outside the red nucleus and rubro-spinal tract were not seriously damaged, although the right nucleus interstitialis was somewhat affected by the needle puncture, a fact which probably accounts for the tendency of the animal to circle to the right. Fibers of the brachium conjunctivum were also involved, of course, as they passed through the red nucleus.

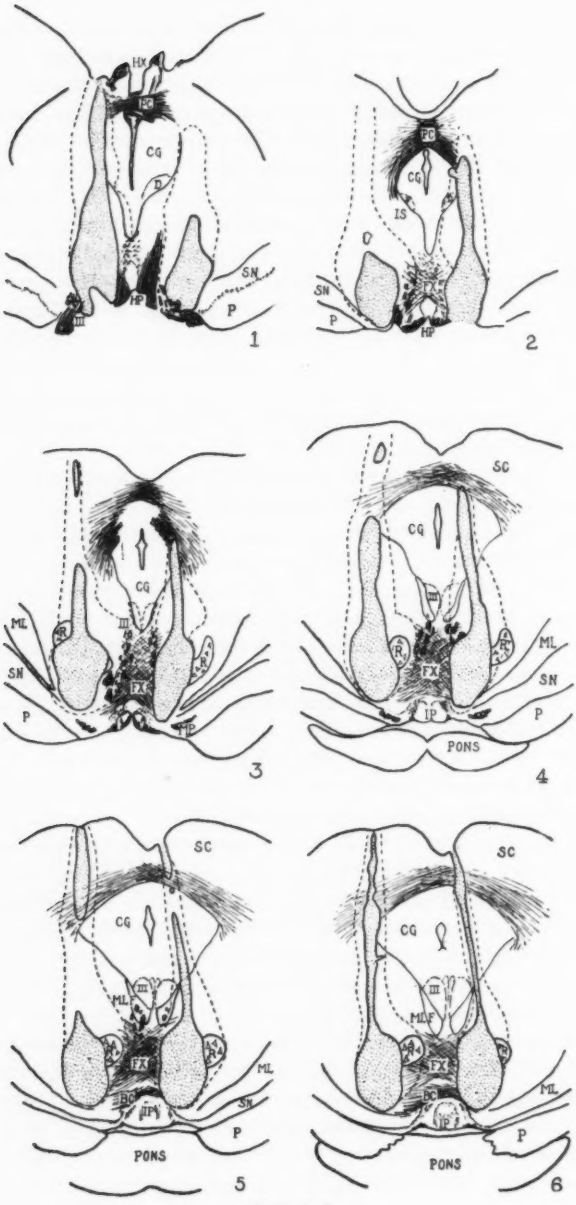
In cat 229 the eyes were enucleated at the time the red nuclei were destroyed. Five hours after the operation this animal was able to right itself from either side, stand, and walk. The gait was awkward and ataxic, and there was pronounced scissoring of the forelimbs. There was a tendency to circle to the right. In the hammock rather marked rigidity was observed, there being a good stütz reaction in each limb, and good resistance to passive flexion in all but the left hindlimb, in which it was slight.

In this case the lesion involved the medial portions of both red nuclei and destroyed the rubro-spinal tracts throughout their course in the mesencephalon. The red nuclei were involved in the edematous zone of the lesion. The interstitial nuclei were partially injured, as was the lateral part of the right medial longitudinal fasciculus. The lesions extended from the caudal pole of the mammillary nucleus to the pons.

Cat 233 was also subjected to enucleation of the eyes at the time of operation. Six hours later it was able to right itself and stand, and, although apathetic and disinclined to activity, walked with an unsteady, ataxic gait. The extensor muscles showed considerable rigidity when the animal was placed in the hammock, the stütz reaction being readily obtainable in each limb. There was fair resistance to passive flexion in the forelimbs, but slight in the hindlimbs.

The lesions in this instance extended from the region just caudal to the mammillary bodies to the pons. The red nuclei were practically completely destroyed and the rubro-spinal tracts interrupted. There was some damage to the left nucleus of Darkschewitsch and nucleus interstitialis.

Cat 235 was able to right itself, even when blindfolded by a mask which covered the head, and could stand. It walked awkwardly, circling to the left, and exhibited considerable dysmetria and ataxia. When suspended,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Figs. 1–6

the forelimbs were extended, the hindlimbs partially so, and a good stütz reaction could be elicited in each leg. There was strong resistance to passive flexion in all but the right hindlimb, where it was slight.

In this instance the lesions also extended from the mammillary bodies to the pons, destroying the medial portion of the left red nucleus, all of the left rubro-spinal tract, and the portion of the decussation to the left of the midline. The lateral portions of the right red nucleus and rubro-spinal tract were destroyed; fibers in the latter, coming from the left nucleus ruber, were of course cut off more proximally by the lesion in the latter. Fibers from the medial portion of the right red nucleus were destroyed in the left rubro-spinal tract. The left medial longitudinal fasciculus was damaged at the level of the oculomotor nucleus, and the left nucleus interstitialis severely affected.

Cat 237 could right itself perfectly when not blindfolded. When masked it was able to get up from the right side, but manifested considerable difficulty in doing so when the left side was down. It stood in awkward postures, and in walking exhibited the incoördination characteristic of the other case. The extensor rigidity observed when the animal was suspended was quite marked; there was a good stütz reaction from each limb, and resistance to passive flexion, although slight, was present.

The lesions extended rostrally from the pons almost to the mammillary bodies, completely destroying the rubro-spinal tracts and inflicting con-

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Fig. 1. Portion of transverse section through the brain stem of cat 231, showing the extent of lesions. The stippled area indicates the lesion; the area included within the broken line showed some slight evidence of edema.

Fig. 2. Portion of transverse section through brain stem of cat 231, taken approximately 0.7 mm. caudal to that in figure 1.

Fig. 3. Portion of transverse section through the brain stem of cat 231, taken approximately 0.5 mm. caudal to that in figure 2.

Fig. 4. Portion of transverse section through the brain stem of cat 231, taken approximately 0.65 mm. caudal to that in figure 3.

Fig. 5. Portion of transverse section through the brain stem of cat 231, taken approximately 0.65 mm. caudal to that in figure 4.

Fig. 6. Portion of transverse section through the brain stem of cat 231, taken approximately 0.1 mm. caudal to that in figure 5.

Abbreviations used in figures

BC, Brachium conjunctivum
CG, Central gray
D, Nucleus of Darkschewitsch
FX, Decussation of Forel
HP, Habenulo-peduncular tract
HX, Habenular commissure
IP, Interpeduncular nucleus
IS, Interstitial nucleus
ML, Medial lemniscus
MLF, Medial longitudinal fasciculus
MP, Mammillary peduncle
P, Cerebral peduncle
PC, Posterior commissure
R, Red nucleus
SC, Superior colliculus
SN, Substantia nigra
III, Third nerve or its nucleus

siderable damage upon the red nuclei themselves. There was probably some injury to the right interstitial nucleus and to the nucleus of Darkschewitsch of the same side.

The two cats which did not walk perhaps deserve brief consideration. One of these, cat 230, was very inert and apathetic following the operation, which included enucleation of the eyes, lying on its side and making no attempt to rise, although it was capable of bringing its head to an upright position. When placed on its feet it could stand for a short time, but could not walk without falling forward with the wrists bent under and the hindlimbs extended. When suspended in the hammock some running movements and occasional generalized convulsive twitchings were observed. There was considerable rigidity, as manifested by high grade stütz reactions and resistance to passive flexion in all the limbs. The lesions in this cat were very large, meeting in the midline, and affecting the area just caudal to the mammillary bodies, the brachium conjunctivum on each side, the habenulo-peduncular tract, a large part of the central gray, the central tegmental fasciculi and the interstitial nuclei as well as the left nucleus of Darkschewitsch. The left red nucleus was damaged in its dorsal portion; the right red nucleus and rubro-spinal tract were entirely destroyed. All of the left as well as most of the right medial longitudinal fasciculus was destroyed.

Cat 232, although unable to walk when first examined, showed considerable improvement in the course of a few hours, and at the time it was killed there were indications that ability to walk would return shortly. However, during the time allotted for the experiment, attempts at walking were unsuccessful, although the animal could stand when placed on its feet. When lying on the floor the head was raised and occasional scratching movements were made as if in an attempt to raise the body. There were signs of forced movement which twisted the head so the right ear was lower than the left. When in the hammock there was marked extension of the limbs, with a good stütz reaction all around, and resistance to passive flexion which was marked in the forelimbs and moderate in the hindlimbs. This cat had also been subjected to enucleation, and it may well be that its failure to move about was due, in part at least, to inability to withstand the shock of the two operations. It is not unusual to find in a group, one or two animals whose stamina is not up to the average. The lesions were rather extensive, destroying the floor of the brain at the points where the habenulo-peduncular tracts reach their most ventral level, and causing general damage to the area just caudal to the mammillary bodies. The central gray, nucleus of Darkschewitsch and nucleus interstitialis on the left were largely destroyed. Both red nuclei were severely affected and the rubro-spinal tracts completely destroyed.

Discussion. It is evident from the results presented here that the

behavior of animals in which the efferent descending connections of the red nuclei have been completely obliterated is not essentially different from that of animals deprived of the part of the red nucleus caudal to the third nerve, as described in a previous paper. Again we find a characteristically peculiar gait, mild extensor hypertonus and retention of ability to assume an upright position. Points at which these observations differ from those of Rademaker have been previously pointed out, but we can offer some corroboration of his work in regard to the increased tonicity of the extensor muscles. The rigidity observed by us is apparently of lower grade than that displayed by Rademaker's animals, a fact which may be in part accounted for by the integrity of the cerebral cortex in our cats. However, Rademaker has pointed out that mechanical destruction of the red nuclei in animals retaining the cortex gives rise to rigidity which is moderate compared with that obtained in decerebrate preparations.

Mussen has reported that an electrolytic lesion in the rostral pole of one red nucleus causes loss of the righting reflexes and a transient decrease in muscle tonus. The complete destruction of both red nuclei in our experiments did not produce these results.

The only way in which we can account for these discrepancies is by offering the suggestion that the amount of tissue involved by the lesions produced by these workers must have been greater than was realized at the time. Spitzer (1924) and Lorente de Nó (1928) believe that the rigidity occurring in Rademaker's cats was due to the cutting of a commissural path which passes between the two vestibular complexes and which ordinarily carries impulses inhibiting extensor tonus. It may be, also, that the loss of ability to stand and walk was due to injury to structures situated farther rostrally. In this connection it is interesting to consider our cat 230, above described, which exhibited no righting or walking reactions, and in which the rubral connections were apparently completely destroyed. Other cats, however, of which the latter was equally true were quite capable of orientation and progression, so we must look elsewhere than the red nucleus for the causes of the debility of cat 230. This animal possessed lesions which were much more extensive than usual, destroying a great portion of the medial part of the tegmentum. We also find involvement of the nuclei associated with the upper end of the medial longitudinal fasciculus and of the latter itself, which is perhaps suggestive. Cats showing partial involvement of these structures may show defects as to progression and righting which are typified by what are commonly called forced movements. In all nine of our animals which have shown tendencies to forced curvature to right or left, autopsy material has indicated that there was injury to either the medial longitudinal fasciculus, the nucleus of Darkschewitsch or the nucleus interstitialis on the side to which the forced movement occurred. May it not be possible that almost total or symmetrical

destruction of these structures will eliminate righting and walking capabilities?

We have been unable to determine satisfactorily the extent of any impairment to the righting reflexes in our walking cats, nor which of these reflexes are responsible for the existing righting reactions. That the optic mechanism does not participate is obvious, but we have not been able to decide whether or not the labyrinthine reflexes are ruled out in animals where the lesions are confined to the red nuclei. Observations of the righting reactions when the cats are held in the air by grasping the pelvis, as suggested by Magnus and Rademaker, have not given satisfactory and consistent results in our hands, perhaps because of the temperamental or constitutional responses of cats to such handling. In any case, these animals are generally able to orient themselves in an efficient manner, and it is certain that righting reflexes are not abolished in blind cats by the destruction of the red nucleus.

That the red nucleus plays a part in the regulation of tonus is indicated by our experiments. However, one must not overlook the possibility of such lesions as herein described involving the connections of the region immediately rostral to the red nucleus in the subthalamus, and we cannot be sure that this may not have been a factor in our results. It is, nevertheless, certain that bilateral destruction of the red nucleus does not produce a rigidity at all comparable to that seen in decerebrate animals, nor sufficient hypertonus to prevent locomotion.

The peculiarities of the gait, typified by dysmetria, poor coördination and ataxia, greatly resemble the effects of cerebellar injury. Lesions such as those necessary for destruction of the red nucleus necessarily involve the fibers of brachium conjunctivum, thus cutting off the best known efferent pathway from the cerebellum. If the rubro-spinal tract carried cerebellar influences caudalward one would also look for such symptoms as have been described here after its destruction. The so-called inferior syndrome of the red nucleus, described in a number of clinical cases, includes similar locomotor disturbances. While the nucleus ruber seems to participate in the regulation of tonus, its classical designation as a center for transmission and selection of cerebellar, cortical and pallidal influences acting on the final common pathway may still be regarded as eminently reasonable.

SUMMARY

1. Complete destruction of the red nuclei or their descending connections in the cat by restricted lesions leaves an animal capable of righting itself, standing, and walking, even after optic influences have been removed. It is therefore evident that the red nucleus cannot be regarded as an absolutely essential center for the righting function in blind animals.

2. The gait of such a preparation is usually characterized by dysmetria,

poor coördination, and certain types of ataxia, which may be due to involvement of the cerebellar efferent pathway.

3. Under suitable conditions these animals display extensor hypertonus of mild degree, which does not significantly interfere with locomotor or postural activities. It is suggested that the red nucleus alone cannot be held responsible for the inhibitory influences the removal of which causes decerebrate rigidity, but that this structure is merely a portion of the complex system regulating postural functions.

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A TECHNIQUE FOR OBTAINING AND RECORDING ISOMETRIC CONTRACTIONS OF MAMMALIAN SKELETAL MUSCLES IN SITU

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In connection with a study of the energy metabolism of individual mammalian muscles in situ data on the isometric tensions developed by the muscles under investigation were required. The following technique was therefore developed.

The gracilis muscle of an anesthetized dog was freed from adjoining tissues with care not to injure its vascular and nervous connections. The tendon of insertion was dissected as far down the knee as convenient, transected, and secured to a specially designed clamp through which it was attached by a stout cord to a torsion wire myograph.

The clamp is illustrated in A, figure 1. The proximal end of the tendon was laid over the flat plate *a*, and the distal portion passed around the bar, *c*, and brought back beyond *a*, where it was secured by screwing plate *b*, firmly against *a*. The tendon was thus "snubbed" around *c*, obviating all danger of slippage when the clamp was properly tightened.

The myograph was constructed after the general design worked out in Sherrington's laboratory (Fulton, 1926). It is illustrated at B in figure 1. The myograph proper consists of a piece of piano wire approximately 1.5 mm. in diameter, *d*, secured immovably at one end to a metal block and resting snugly for 12 mm. of its length near its opposite end in a semi-cylindrical groove (which it fits exactly) in another metal block similar to the first. The length of wire between the blocks is exactly 50 mm. and the attachment to the muscle is exactly 5 mm. from the grooved block. Both blocks are fixed on a metal base which, in turn, slides in grooves in a metal frame, as indicated in B. The adjustment screw, *e*, allows the slack on the cord to be taken up and the initial tension of the muscle to be set as desired. The method of transmitting the torsion of the wire to a writing point is indicated in diagram C. The magnification used was about 50-fold. The resistance of the torsion wire was such that a tension of 4000 grams gave a record about 35 mm. in height. The myograph was carefully calibrated in grams per millimeter rise of the recording lever.

To avoid errors due to shifts in the position of the origin of the muscle

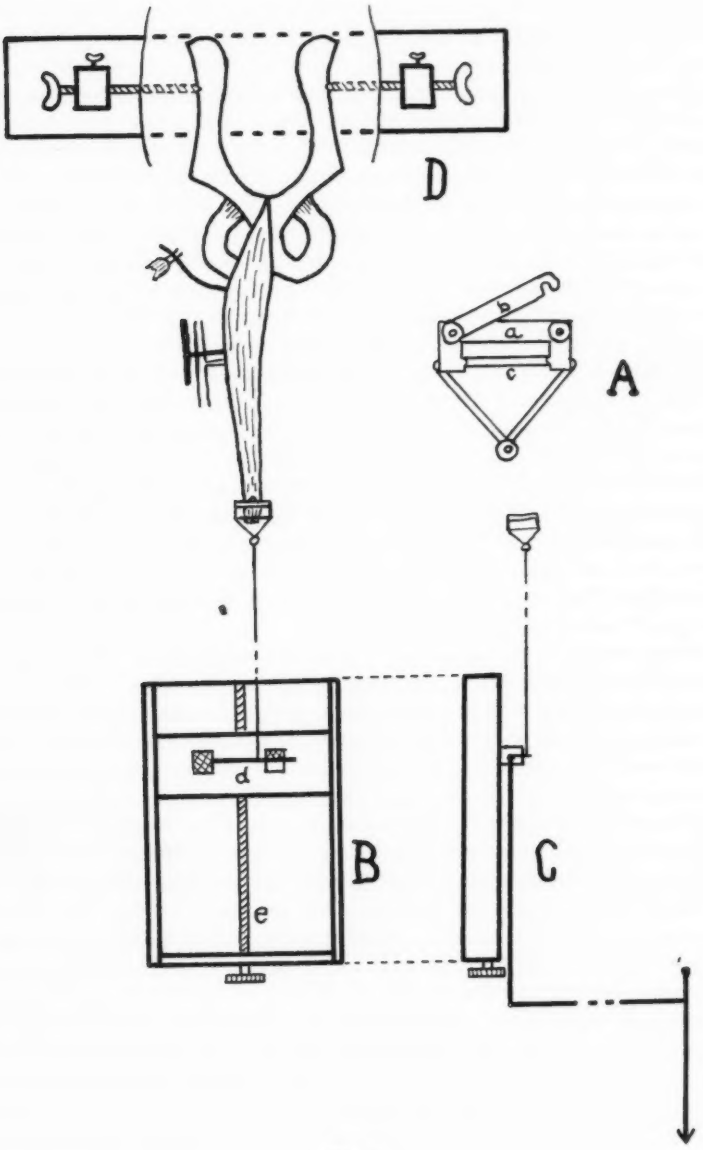

Fig. 1

the pelvis and leg were carefully immobilized. In the early experiments, modifying a technique of Liddell and Sherrington (1923), the pelvis was immobilized by passing a steel drill, 0.5 cm. in diameter and 45 cm. in length, transversely through both ilia, and securing the ends of the drill to the dog board. Satisfactory immobilization of the pelvis was thus secured, but we so frequently had the misfortune to damage an artery and lose considerable quantities of blood, besides finding it necessary to open the abdomen to tie off the ruptured vessel, that we devised a means of obtaining immobilization without penetrating the pelvis. The arrangement is shown at D, figure 1. It consists of a brass plate 45 cm. long by 10 cm. wide, on which are mounted two brass blocks, 20 cm. apart. Threaded bolts pass through the blocks as shown in D. These are 4.5 cm. above the base, and are pointed at their inner ends.

The procedure of immobilization was briefly as follows: With the dog in position on the board (a flat dog board was used in all this work) the outer surfaces of the two ilia were made accessible by incising the skin and overlying muscles. The brass plate was placed directly under the pelvis as indicated in the diagram. With the aid of guidance by the fingers the two bolts were screwed toward each other till their pointed tips engaged and held the two ilia. The bolts were then fixed in position by means of set screws, and the plate clamped to the dog board. With the pelvis thus immobilized the leg was drawn into line with the long axis of the body and tied firmly in place at knee and ankle.

Before attaching the insertion of the gracilis muscle to the myograph preparations were made for obtaining metabolic data (for details see accompanying paper); the gracilis branch of the obturator nerve was also exposed and a pair of shielded electrodes (vulcanite type) applied. The muscle was then attached to the myograph. The base carrying the torsion wire was screwed away from the muscle till all slack was out of the connecting cord and a slight initial tension (not exceeding 20 gm.) established.

Tetanic stimuli were applied to the nerve, the inductorium used being of the small Harvard type. Except in a few experiments the strength of stimulation was clearly supramaximal for the nerve trunk. The stimuli were interrupted rhythmically, periods of stimulation 0.5 second in duration alternating with periods of non-stimulation 0.7 second in duration, giving thus a stimulation rhythm of 50 per minute. This rhythm was selected as constituting a form of activity comparable to some types of maximum human exertion (in mounting stairs at top speed, four steps at a time, individual muscles contract about once a second and are probably in contraction about 0.35 second at each effort).

The primary purpose of the experiments was to obtain metabolic data (see accompanying paper) consequently the work periods were discontinued as soon as enough blood was collected to permit of determinations of oxy-

gen and sodium lactate concentrations in the blood. The work periods were therefore not of equal length, ranging between 0.5 minute and 3.0 minutes and averaging 1.3 minutes. Since the muscles contracted 50 times a minute the average number of contractions was 65. Satisfactory tension records were obtained in 39 experiments. In all of them the tension developed in response to stimulation fell off as the work period progressed, shading in the later stages more or less gradually into a "steady state." (In six experiments the contractions became too weak to move the writing lever to a measurable extent before a steady state was reached.) In twelve experiments the first dozen contractions (on the average) were of equal height or showed a definite "treppe," the later contractions falling off as described; in ten experiments the rate of decline was slow at first and subsequently became greater; in fifteen the rate of decline was strikingly uniform except for the shading into the steady state described above; in only two was the decline more rapid in the early stages of the period than later. The tension development during the steady state was at different levels in the different experiments, but the latter fell into two quite definite groups according to the amount of difference between the steady state and the initial activity. In the first group the tension development during the steady state averaged about 25 per cent of the initial maximum, while in the second group it averaged only 10 per cent of the maximum. The trend of activity was therefore either to a steady state in which individual contractions tended to be about one-fourth as powerful as the maximum of which the muscles were capable, or to a steady state in which the intensity of contraction averaged about one-tenth the maximum, or to a condition of complete exhaustion. The first trend appears to us more nearly typical of muscles functioning normally within the body for reasons which are presented in the accompanying paper.

Three alternatives suggest themselves as means of accounting for the decline in tension development. The first is the dropping out of motor units (Sherrington, 1925) through failure of excitation of some motor axons; the second is the dropping out of some of the muscle fibers within individual motor units through the onset of local "fatigue" of myoneural junctions; the third is fatigue of the muscle fibers themselves. As a matter of fact we have evidence that more than one of these factors may actually interact to bring about decline in developed tension. We have observed that a shift of the stimulating electrodes to a fresh area of nerve sometimes results in improvement in performance, proving at least part of the decline, in these cases, to be due to failure of excitation of some of the motor neurons, presumably through local "polarization" effects. We have not seen the initial tension fully regained by this procedure, consequently one or the other intramuscular factor must also operate to reduce the later contractions below the intensity of the earlier ones.

We plan to present in a subsequent communication some observations and interpretations bearing on the contractile powers shown by mammalian skeletal muscles in situ. The present paper is therefore confined to matter that is considered germane to the substance of the accompanying paper on the activity metabolism of muscles in situ.

SUMMARY

A technique is described whereby isometric contractions of dogs' gracilis muscles stimulated in situ through their nerves are recorded by means of a torsion wire myograph of special design.

Periods of activity composed of tetanic contractions of 0.5 second duration at a rate of 50 per minute, show a progressive decrease in tension development either from the first or after about a dozen contractions.

The trend of tension development is either toward a "steady state" in which the muscle continues to develop an approximately constant tension during a long series of contractions or toward a condition of "exhaustion" in which not enough tension is developed to be measured on the tracing.

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THE ACTIVITY METABOLISM OF MAMMALIAN SKELETAL MUSCLE IN SITU[1]

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The studies of muscle metabolism which have been in progress in this laboratory since 1926 have been concerned primarily with the processes in which production of lactic acid is a predominant feature. The interpretation placed upon these processes has recently undergone profound modification, consequent on the discovery of creatine phosphoric acid (Fiske and Subbarow, 1927; Eggleton and Eggleton, 1927) and of the occurrence of normal contractions in muscles poisoned with monoiodoacetic acid without any concurrent production of lactic acid (Lundsgaard, 1930), but for the basic problem of relating the chemical energy turnover occurring in muscle in situ to the potential or actual yield of mechanical work, studies of oxygen consumption and lactic acid production continue to constitute the most logical and practical method of attack. The precise thermodynamic determinations of A. V. Hill have been carried out mainly on excised muscles of the frog. Meyerhof's chemical studies have been made chiefly on similar material. We have performed our experiments on anesthetized dogs with circulation through the muscles studied as nearly unimpaired as possible.

METHODS. As in our work previously reported (Martin, Field and Hall, 1929) amytal anesthesia was used. Continuous records of arterial blood pressure were obtained from one carotid, using an ordinary mercury manometer. Continuous records of oxygen consumption were also made with the aid of a Sanborn "grafic" metabolism apparatus. Readings of body temperature were taken at sufficiently frequent intervals to enable us to plot a continuous approximate record of body temperature.

The experiments to be reported in this paper fall into three groups, according to the treatment to which the muscles were subjected. The first group (HBl) includes experiments in which no activity was induced. In

[1] A preliminary abstract report of part of this work appeared in this Journal, 1931, xcvii, 545.

[2] We desire to acknowledge the valuable assistance rendered at various stages of this investigation by the following students: J. Crismon, R. Eklund, S. M. Field, R. Goodcell, F. Harvey, S. Hayes, M. Jameson, M. Miller, R. Skow, D. Storey, and R. Vickers.

the second group (KL) a single muscle (gracilis) was worked in situ by rhythmic excitation of the gracilis branch of the obturator nerve. In the experiments of the third group (J) the main muscles of one leg were worked similarly, stimulating electrodes being placed on the femoral, sciatic and obturator nerves, each of which was sectioned central to its electrodes.

In all the experiments the muscles whose lactate content was to be determined were prepared by separating them from adjoining tissues as fully as possible without disturbing any vascular or nervous connections and without cutting any attachments. This work was done early in the experiments; the muscles were then covered with gauze soaked in warm Ringer's fluid and the skin drawn back into place. At the desired moment the test muscles were frozen in situ with carbon dioxide snow according to a method suggested by Davenport and Davenport (1928) and were then excised and thrown into liquid air. (In some of the experiments the CO_2 snow freezing was omitted from one or more muscles for purposes of comparison.) The muscle substance, frozen in liquid air, was ground finely in a mortar; five grams were then weighed out and suspended in water. Proteins were precipitated by the copper sulphate tungstate method (M. Somogyi, 1931) and carbohydrates by the method of Van Slyke (1917). The subsequent analytical treatment was the same as used by us for the determination of blood lactates (Martin, Field and Hall, 1929). Arterial blood samples were taken through a cannula placed in the carotid not in use for the arterial pressure record. These were analyzed for lactates by the procedure indicated above.

Blood and muscle lactates in resting dogs. To assure ourselves that observed changes in lactate content following activity were properly attributable to the activity and not merely consequent on the passage of time the experiments of our first group were done. The only operative procedures additional to those previously described consisted in exposing one brachial and one femoral vein, and in some of the experiments still other large veins, to permit the withdrawal of samples of venous blood, usually by simple venopuncture with syringe needles.

About ten minutes were allowed to elapse after completing the operative preparations to permit the records of arterial blood pressure and oxygen consumption to get well under way. Samples of blood (about 5 cc. in volume) were then drawn from an artery, a brachial vein and a femoral vein, and one or sometimes two flexor muscles of the foreleg were frozen in situ and excised. These tasks were completed within about fifteen minutes ordinarily. A further wait was then allowed of fifteen minutes to a half-hour, followed by freezing and excision of one hind leg muscle (gracilis or rectus femoris; in a few instances a gastrocnemius). In about half the experiments a fore leg muscle was also excised at this time. A further period of at least a half-hour was then allowed to pass. The third and final

set of samples was then taken, including hind leg muscles in nearly all the experiments, fore leg muscles in a few, and arterial and venous blood samples in nearly all.

Our findings in respect to lactate content are summarized in table 1. They show quite clearly that no great changes in either blood or muscle lactates occur in amytalized dogs during the period, usually not exceeding an hour and a half, occupied by our activity experiments of the subsequent groups.

Some incidental findings are of enough interest to justify brief comment. It will be noted that the lactate contents of brachial vein blood and femoral

TABLE 1

Muscle and blood lactates in resting dogs

All figures in milligrams per cent lactic acid.

	MEAN	RANGE
Early samples		
Arterial blood	24.4*	*6.8–52.5*
Femoral vein blood	21.0	*10.8–33.0*
Brachial vein blood	23.6	*11.6–59.0*
Flexor muscle of fore leg	48.4*	*15.6–90.6*
Samples one-half to one hour later		
Fore leg muscles	35.0	*19.1–46.4*
Hing leg muscles	40.7	*23.7–63.2*
Samples more than one hour after the first		
Arterial blood	29.6	*13.3–46.8*
Venous blood	35.4	*16.9–76.0*
Fore leg muscles	43.5	*28.6–59.2*
Hing leg muscles	56.5	*14.0–86.0*

* Data from the entire series of experiments are included.

vein blood averaged about the same. This is neither a novel nor an unexpected observation, but is worth recording as supporting the idea that the basal metabolic processes in muscles in different regions tend to be pitched on about the same scale. In general high arterial blood lactates are associated with high venous blood lactates and both with high muscle lactates. The correspondence is less close, however, than would perhaps be anticipated, the Pearson coefficient of correlation between arterial and venous blood lactates being only 0.75 $\pm$0.046; between venous blood lactates and muscle lactates 0.70 $\pm$0.055, and between arterial blood lactates and muscle lactates 0.060 $\pm$0.064. In assembling the above data none

were paired unless they were obtained from samples taken within a few minutes of each other. For the correlation between arterial and venous lactates the mean of the values obtained from brachial and femoral vein bloods was taken as representing the approximate composition of mixed venous blood. For correlating muscle lactates with venous blood lactates fore leg muscles were paired with brachial vein blood and hind leg muscles with femoral vein blood.

The occasional lack of correspondence between arterial and venous blood lactates noted above has been pointed out by other workers (Himwich, Koskoff and Nahum, 1930) and we have considered one aspect of it in another place (Martin, Field and Hall, 1930a). We were led by it to perform a few experiments in an attempt to locate the points in the circulation at which bloods with quite different lactate contents become intermingled. Our argument was that if the blood in the femoral vein, for example, has a high lactate content, while arterial blood has a low lactate content, not only must the hind leg muscles be contributing lactates to the blood, but at some point in the course of the blood from the femoral vein toward the left ventricle it must become diluted with blood of a decidedly lower lactate content. (The possibility that the heart muscle may withdraw lactates directly is also not to be overlooked.) A practical difficulty confronting us in this work is that we have no means of knowing during individual experiments whether the arterial and venous lactate values are near together or far apart, the lactate analyses not being completed until some hours after the acute experiments are over.

In these experiments we drew venous blood through a well vaselined male urethral catheter, which was passed into the venae cavae by way of the right external jugular vein. Careful measurements were made on the basis of which we estimated how far the catheter must be inserted to bring its tip successively into the superior vena cava, the inferior vena cava above the entry of the hepatic vein, the same between the hepatic and renal veins, and finally the same below the entry of the renal veins and above the bifurcation. All locations were checked post mortem in each experiment and found to have been successfully obtained. Our most striking experiment was one in which the lactate content of arterial blood was 15.5 mgm. per cent, while blood from the inferior vena cava below the renals showed a lactate content of 42.2 mgm. per cent. The sample from the same vein above the renals and below the hepatic had 35.1 mgm. per cent lactate; the sample from above the hepatic, 19.3 mgm. per cent, and the sample from the superior vena cava 20.9 mgm. per cent. In this experiment the liver was certainly contributing blood of low lactate content. Whether it was actually withdrawing lactates from the blood as Himwich and co-workers have shown it to do in many cases (loc cit., 1930) cannot be stated with certainty from our data, although the presumption is that

it was doing so. In another experiment the lactate content of arterial blood was 15.8 mgm. per cent and of inferior vena cava blood from below the renals 23.0 mgm. per cent. A sample of blood taken directly from a renal vein contained 29 mgm. per cent lactic acid, and blood from the inferior vena cava above the hepatic 27.5 mgm. per cent. However, blood from one brachial vein contained 15.3 mgm. per cent lactate, and from a subclavian vein only 9.6 mgm. per cent. In this case the forward end of the body was contributing the blood of low lactate content by which the arterial blood took on a lower lactate value than postcaval blood. In four other experiments there proved to be no significant differences in lactate content between arterial blood and blood from the inferior vena cava at the different levels from the bifurcation forward.

TABLE 2

Data on oxygen consumption, rectal temperature and arterial pressure from the experiments of the first group (19 experiments)—resting dogs

PERIOD	OXYGEN CONSUMPTION PER KILOGRAM PER MINUTE		RECTAL TEMPERATURE		ARTERIAL PRESSURE	
	Mean	Range	Mean	Range	Mean	Range
1*	5.4	*2.6–7.5*	37.6	*34.0–39.8*	99	*56–158*
2	5.3	*3.8–7.0*	37.7	*34.6–41.2*	93	*70–125*
3	5.3	*3.9–7.2*	37.6	*34.6–41.6*	86	*62–123*
4	5.3	*3.8–6.5*	38.2	*35.0–41.6*	82	*64–113*

The periods represent half-hour intervals.

The first period began immediately upon completion of the operative preparations. It varied from one hour to about two and one-half hours after administration of amytal.

* The data of the first period include all the experiments reported in this paper. The mean values for the experiments of the first group taken by themselves agree closely with those in the table although the ranges differ slightly.

Our observations on oxygen consumption, rectal temperatures and arterial blood pressures in the experiments of this group are summarized in table 2. No special comment is necessary except in connection with the arterial pressure data, which show a steady decline in arterial pressure with the passage of time. The experiments described in this paper did not exceed two hours in length after the starting of the records of arterial pressure, and within this period the decline does not assume significant proportions; but it is clear that in prolonged experiments in which amytal is used as anesthetic possible effects of the drug on arterial pressure should be taken into account.

Metabolic data from single muscles. In the experiments of the second group (KL) the gracilis muscle of each side was separated from its adjoining tissues, the gracilis branch of the obturator nerve was exposed, and also

the femoral artery and vein for a short space both above and below the origin of the main nutrient vessels of the muscle. Great care was taken to avoid injury to or compression of these latter. All minor veins in the exposed area, other than the one communicating with the gracilis muscle, were tied off. A cannula directed centrally was inserted into the femoral vein just below the point of union of the gracilis vein. The cannula and femoral vein up to the gracilis vein were then filled with a thin colorless oil (petrolatum liquidum).

After the above preparations were complete the pelvis and one leg were carefully immobilized and the tendon of the gracilis muscle on the immobile side was cut and attached to a torsion wire myograph (for details of the latter and of the technique of immobilization see accompanying paper).

After these preparations were finished a well vaselined syringe was attached to the cannula in the femoral vein and at a given signal the same vein was clamped off central to the point of entry of the gracilis vein. All the blood entering the femoral vein from the gracilis muscle was thus diverted into the syringe, and the total resting blood-flow for any desired short period collected. No special effort to avoid clotting was used other than the well greased cannula and syringe. The withdrawal of blood was continued until enough was obtained for determinations of oxygen content (by the method of Van Slyke) and of blood lactate content. The volume and time were carefully noted. The volumes averaged 10 ml. and the periods 3 minutes. The muscle was then worked by administering 50 brief tetanic stimulations per minute to the gracilis branch of the obturator nerve (for details see accompanying paper). The resultant isometric contractions were recorded on a slowly moving drum. During the work period the venous blood from the muscle was collected in the same manner as described above. Our practice was to continue the stimulation until as much blood was obtained as needed for the oxygen and lactate determinations. In some cases thirty to forty-five seconds sufficed, in two or three nearly three minutes were required; the majority were worked 1 to 1.5 minutes.

At some convenient stage in the proceedings, usually just before withdrawing the first sample of resting venous blood, a sample of arterial blood was taken, and the rectus femoris muscle on the same side was frozen in situ with carbon dioxide snow, excised and ground to powder in liquid air to be analyzed as a control on the lactate content of the resting gracilis muscle. Immediately upon completion of the stimulation the worked gracilis (whose length had been measured in the meanwhile) was similarly treated and excised. The portions of muscle not used for analysis were collected and the weight of the whole recorded. All these procedures were then repeated on the opposite side of the body.

At the end of each experiment the following data were in hand: the lac-

tate content and oxygen content, first of arterial blood, second, of the venous blood circulating in a known time through a resting gracilis muscle of known length and weight, and third, of all the venous blood that passed through the same muscle during a known brief period of work; also the volume of blood-flow during a known resting period and the entire work period; also the lactate content of the gracilis muscle at the end of the work period; and to give an idea of the lactate content of the gracilis prior to stimulation, the corresponding datum from an adjoining (rectus femoris) muscle in the resting state. The tension developed by the worked muscle at each contraction and the total number of contractions were also known. The quantities of oxygen present in blood were expressed in cubic centimeters per 100 ml.; of lactate in blood in milligrams lactic acid per 100 ml.; and of lactate in muscle in milligrams lactic acid per 100 grams of muscle tissue.

When attempts were made to summarize the metabolic data in tabular form we encountered the fact (considered in detail in the accompanying paper) that in every experiment the tensions developed in successive contractions declined more or less rapidly from a maximum registered at or near the beginning. It is obvious that the metabolism of the active muscles must also have declined as the work continued, and more or less parallel with the decline in developed tension (see p. 490). In view of this decline, and also of the variation in length of the work periods in the different experiments the findings cannot be satisfactorily summarized in terms of the usual arbitrarily selected time unit, of one minute, but must be presented rather in terms of the time that actually elapsed in the work periods. This time ranged from 0.5 minute to 3.0 minutes, averaging 1.3 minutes. Table 3 accordingly presents our metabolic data averaged in terms of this mean work period. The gracilis muscles studied in these experiments ranged in weight from 12.5 grams to 80.0 grams, averaging 44.0 grams. In length the range was from 9 cm. to 21 cm., with an average of 16 cm. The maximum tension developed by individual muscles ranged from 1810 grams to 6200 grams, averaging 3400 grams.

Metabolic data from experiments in which one hind leg was worked. In our third group of experiments the main muscles of one hind leg were stimulated as described on page 482. Control samples were taken from the opposite hind leg. The series is short, consisting of but nine experiments. The data on blood and muscle lactates are summarized in table 4. Neither the rectal temperature nor the arterial blood pressure were significantly altered by the amount of work given these dogs. The mean pre-work temperature was 37.3°C.: the mean at the end of activity 37.5°C.; and the mean for the succeeding half-hour period 37.2. The mean arterial pressures were: pre-work, 105 mm. Hg; during work, 100 mm. Hg; during the succeeding half-hour period, 92 mm. Hg.

The oxygen consumption of the whole animal (table 5) increased de-

cisively in every experiment within a few seconds of the beginning of stimulation, and the consumption rate rose rapidly to its maximum (within the limit of error of the determinations). In two of the experiments (4 and 7R) the increase in oxygen consumption was disproportionately large, and

TABLE 3

Metabolic data from single gracilis muscles worked in situ (50 experiments)

Mean work period = 1.3 minutes

	MUSCLE AT REST		MUSCLE WORKED	
	Mean	Range	Mean	Range
Blood flow per 100 grams muscle per minute	9.0	*4.0–21.8*	21.5	*11.3–46.0*
Blood flow per 100 grams in 1.3 minutes	11.8		28.0	
Oxygen content of arterial blood (cc. per 100 ml.)	20.0	*12.8–25.4*	20.0	
Oxygen content of gracilis vein blood (cc. per 100 ml.)	9.5	*3.1–16.2*	6.8	*1.6–12.8*
Mean arterio-venous oxygen difference	10.5		13.2	
Mean oxygen consumption per 100 grams muscle in 1.3 minutes	1.2 cc.		3.7 cc.	
Excess oxygen consumption of 100 grams muscle due to 1.3 minutes' work			2.5 cc.	
Lactic acid content of arterial blood (mgm. per cent)	29.0	*14.3–56.7*	29.0	
Lactic acid content of gracilis vein blood (mgm. per cent)	30.0	*15.4–48.9*	37.6	*20.4–69.1*
Amount of lactic acid entering 28.0 ml. blood (mean blood-flow of worked muscle)			2.4 mgm.	
Lactic acid content of gracilis muscle (mgm. per 100 grams)	60.0*	*34.1–133.0*	160.0	*64.4–284.0*
Increase in lactic acid per 100 grams muscle during work			100.0 mgm.	
Total production of lactic acid in 100 grams muscle due to 1.3 minutes' work			102.4 mgm.	

* From rectus femoris muscles taken as controls.

while we do not doubt that the increase was as genuine, and as much due to the stimulation in these as in the other experiments, it seems that a more nearly typical picture can be constructed if we omit them from our averages, which we have accordingly done. The means obtained from our data indicate, therefore, that vigorous rhythmic tetanization of the muscles of

one hind leg in dogs with an average weight of 18.4 kgm. will tend to increase the oxygen consumption from an average of 99 cc. per minute (5.4 cc. per kgm. per min.) to an average for the first three-minute period of 156

TABLE 4

Muscle and blood lactates in experiments in which the muscles of one hind leg were given rhythmic indirect stimulation for 15 minutes

All figures in milligrams per cent lactic acid.

	ARTERIAL BLOOD LACTATES		FEMORAL VEIN BLOOD LACTATES (WORKED SIDE)		FEMORAL VEIN BLOOD LACTATES (NON-WORKED SIDE)	
	Mean	Range	Mean	Range	Mean	Range
Before work	27.1	*19.7–33.8*	29.8	*19.1–43.5*	32.2	*20.4–43.4*
At the end of work	45.0	*35.4–55.4*	56.5	*45.8–74.0*	40.7	*38.0–43.3*
1 hour after work	38.0	*30.0–49.2*	42.0	*31.2–51.5*		

Muscle lactates

	WORKED MUSCLE		NON-WORKED MUSCLE	
	Mean	Range	Mean	Range
Before work			68.5	*58.2–78.0*
At end of work	148.0	*96.0–253.0*	80.0	*69.0–91.0*
1 hour after work	84.0	*77.8– 89.0*		

TABLE 5

Oxygen consumption data from dogs in which all main muscles of one hind leg were worked

	MEAN	RANGE	
Weight of dogs	18.4 kgm.	*14.5–21.0*	kgm.
Pre-work oxygen consumption	99	*80 –130*	(cc. per minute)
Oxygen consumption during work:			
1st three minutes	156	*130 –201*	(cc. per minute)
2nd three minutes	134	*109 –171*	(cc. per minute)
3rd three minutes	131	*108 –166*	(cc. per minute)
4th three minutes	127	*93 –157*	(cc. per minute)
5th three minutes	121	*88 –147*	(cc. per minute)
Oxygen consumption following work:			
1st half-hour	100	*77 –120*	(cc. per minute)

cc. per minute. The maximum oxygen consumption is reached practically at the beginning of the activity, and too soon, so far as we can judge, for any secondary factors to be brought into action through the circulating blood in parts of the body outside the active leg. We therefore attribute

the whole of the early increase in oxygen consumption to the muscles actually thrown into contraction by the stimulation. The weight of these muscles was determined directly in one dog (J4) immediately after the death of the animal, and found to be approximately 1500 grams. This dog weighed 26.3 kgm. In a dog weighing only 18.4 kgm., which is the mean on which our calculations are based, the corresponding muscles can hardly weigh more than 1200 grams. It would appear, therefore, that the immediate increase in oxygen consumption amounting on the average to 57 cc. per minute must be due wholly to the activity of not more than 1200 grams of muscle tissue, corresponding to about 4.7 cc. per 100 grams of muscle per minute. It will be noted that this is a considerably larger average oxygen consumption than we found in our experiments with single muscles. The interpretation of this difference is considered in the discussion.

Discussion. *The partition coefficient for lactates.* The experiments of the first group (muscles not worked) call for no particular discussion beyond mention of the fact brought out in table 1, and reappearing in tables 3 and 4, that the lactic acid content of resting muscles tends to be about double that of the venous blood emerging from them. The same ratio also appears in experiments on worked muscles in which enough time has elapsed to permit equilibrium to be reëstablished (table 4). A coefficient of 2 can therefore be considered to express the partition ratio for lactates between the muscles and blood of dogs.

The metabolic basis of brief tetanic contractions. In the experiments of the second group in which single muscles were worked in situ the primary problem is to relate the oxygen consumption and lactic acid production to the initial energy-yielding process, now generally conceived to be the explosive decomposition of phosphocreatine (creatine phosphoric acid). The muscles were subjected to successive brief tetanizations of equal duration, the stimulation rate of each tetanus being rapid enough to ensure complete fusion of the component contractions. The work of Hartree and Hill (1921) and of Feng (1931) permits the generalization that in unfatigued muscles stimulated thus the tensions developed in the successive contractions bear a constant ratio to the initial heats, which, in turn, are conceived to be directly proportional to the quantity of phosphocreatine decomposed (Hill, 1932). The relative tensions developed in the successive isometric contractions of a given work period may therefore be considered indicative of the relative amounts of phosphocreatine decomposed during the same contractions, at least while the muscle remains unfatigued. In the later stages of work periods, after marked fatigue has developed, the relationship is in question, Feng (loc. cit.) having shown that when fatigue is present, slowing the response of the muscle, the ratio of tension to initial heat rises. According to Bronk (1930), however, the rise in the ratio is seen only in

experiments in which the frequency of stimulation is such that fusion is incomplete prior to the onset of fatigue and becomes complete as fatigue develops. Feng appears to consider his results comparable to and confirmatory of Bronk's, consequently the greater economy of contraction of fatigued as compared with fresh muscles cannot be considered to be established for all stimulation frequencies, and may conceivably not apply to the conditions of our experiments, in which complete fusion was present at all stages.

Disregarding for the present this possible consequence of fatigue we may therefore analyse our experiments on the principle that the relative tensions developed in the successive isometric contractions of a given work period are reliable indices of the relative amounts of phosphocreatine decomposed in the same contractions.

The resynthesis of phosphocreatine. Virtually coincident with the decomposition of phosphocreatine is the beginning of its resynthesis. In its first stages this resynthesis is largely or wholly oxidative, provided oxygen is available (Eggleton and Eggleton, 1928). In the absence of oxygen, or if the supply of the same is insufficient, a supplementary process involving lactic acid production comes into play (see Hill, 1932, for summary and citations). That portion of the resynthesis which depends on lactic acid production appears to be slower than the oxidative portion in that, in frog's muscles, production of delayed anaerobic heat sometimes continues for as much as three minutes after contraction is over (Hartree and Hill, 1920). The total energy of the restorative reactions is slightly in excess of the energy cost of resynthesizing the decomposed phosphocreatine, as shown by the invariable liberation of a small quantity of delayed anaerobic heat as just indicated, but is nearly enough equivalent to justify the conclusion that the restorative reactions are governed either directly or indirectly by the decomposition of phosphocreatine.

The energy-magnitude of the restorative reactions attending any isometric contraction may therefore be looked upon as in direct ratio to the quantity of phosphocreatine decomposed, which in turn is in direct ratio to the tension developed. The last thus serves as an index to the first, entitling one to interpret data on oxygen consumption and lactic acid production in terms of the relative tensions developed during the successive contractions of a work period.

Delayed lactic acid production in mammalian muscles. The ratio considered just above is valid only for the sum total of the restorative reactions, hence is applicable to cases in which lactic acid production is involved only when the delayed production is included along with the immediate production. It becomes necessary, therefore, to account for the delayed production in order to use tension development as an index of metabolism. We offer the following considerations as showing that the delayed acid pro-

duction is accounted for in our work. The argument depends for its validity on three assumptions, all of which have evidence, either direct or indirect, in their support. These assumptions are: first, that in mammalian muscles at body temperatures the delayed acid production is not so long drawn out as in frog's muscles, but ends within not much more than one minute at the most instead of requiring three or thereabouts; second, that the act of tension development has no effect *per se* on the production of lactic acid, so that the incidence of subsequent contractions does not affect the delayed production of acid consequent on earlier contractions except as such production is influenced in general by the quantities of decomposed phosphocreatine and of lactic acid present in the system; third, that oxidative resynthesis of phosphocreatine takes precedence over resynthesis due to acid production, and to such an extent that in the presence of adequate oxygen little or no production of lactic acid takes place.

If these assumptions are sound the determinations of lactic acid given in tables 3 and 4 include the delayed as well as the immediate production, and therefore give valid data on which to base interpretations of tension records. This conclusion follows from the fact that in an average work period, such as must be taken as the basis on which to interpret mean metabolic data, the developed tension falls off markedly during the later stages (see accompanying paper). The decomposition of phosphocreatine declines in parallel ratio, reducing correspondingly the accretion of additional amounts requiring resynthesis. The oxygen supply becomes more and more nearly adequate to care for these accretions as they become progressively less (see below), thus the need for additional immediate production of acid falls well toward or quite to zero. The power of the muscle to produce lactic acid is not, however, exhausted (in no case have we seen in muscles worked by successive brief tetanizations as high concentrations of acid as were observed by Eggleton and Evans (1930) after continuous tetanization for several seconds). Hence the delayed production referable to the early part of the work period, when the requirement for acid is at its peak, is to be presumed to be largely met during the later part of the period; meanwhile the delayed production referable to the later part has become so small that the time required for chilling the muscle with carbon dioxide snow (amounting to at least a minute) is adequate to permit all or virtually all of the residual delayed acid production to take place. We conclude, therefore, that our worked muscles, when analyzed, contain the whole amount of lactic acid called for by the quantity of phosphocreatine decomposed.

The theoretical oxygen requirements in brief tetanic contractions. In the experiments under consideration an average work period included 65 tetanic contractions, which were of unequal value (at least after the first few), becoming progressively less powerful as the work proceeded until either

exhaustion or a "steady state" was reached. If the contractions had all been of equal intensity (and assuming no change in "efficiency" to have occurred) one sixty-fifth of the total oxygen consumption and an equal proportion of the total lactic acid production would properly be attributable to each contraction. Table 3 shows that in an average work period 100 grams of muscle used 2.5 cc. of oxygen (above the resting requirement) and produced 102.4 mgm. of lactic acid. The proportional share for each contraction would therefore be 0.038 cc. of oxygen and about 1.55 mgm. of lactic acid. According to the calculations of A.V. Hill (1926, p. 88) the removal of 1 mgm. of lactic acid by oxidation implies the consumption of approximately 0.143 cc. of oxygen. If sufficient oxygen could be consumed during work to support the entire energy metabolism, the amount corresponding to the above lactic acid production would be about 0.222 cc. This, added to the amount consumed directly, gives a total of 0.26 cc. as the "net theoretical oxygen requirement," or net metabolic cost, of a single average contraction of 100 grams of muscle in the experiments of this series. The oxygen requirement of the resting muscle for the period of a single contraction cycle is about 0.02 cc. Hence the "total theoretical oxygen requirement" for a single average contraction is about 0.28 cc. per 100 grams of muscle. At the contraction rate used in our experiments this corresponds to a theoretical requirement of 14 cc. of oxygen per 100 grams per minute.

Our first step in comparing the metabolic costs of individual contractions with the metabolic cost of the average contraction of the series was to determine to what extent the greatest contraction of a given work period exceeded the mean contraction of the same period. In 21 experiments the ratio of greatest tension to mean tension ranged between 1.20 and 2.16, averaging 1.5. Since the average "net theoretical oxygen requirement" was 0.26 cc., this implies a "total theoretical oxygen requirement" for the contractions of greatest intensity of the order of 0.41 cc. This theoretical requirement was met in part by a consumption of oxygen during the contraction (and the immediately following brief period of relaxation) and in remaining part by excess production of lactic acid, some of which occurred during the period of the contraction and some of which was delayed. A method of deducing the immediate oxygen consumption is afforded by an analysis of the blood supply. We accordingly turn to such an analysis at this point.

The blood supply of individual fibers. A study of the capillary circulation in the gracilis muscles of dogs has recently been made in this laboratory (Martin, Woolley and Miller, 1932). The conditions of rest and activity of the test muscles were purposely made to correspond as closely as possible with the corresponding conditions in the muscles studied in the present series, hence the findings in that study are fully applicable to this. The

study shows that in the resting muscle at any given instant about sixty per cent of the fasciculi have open capillaries, and that within each fasciculus with open capillaries the ratio of number of muscle fibers to number of open capillaries is about three to two. It follows that in the muscle as a whole the effective ratio of fibers to open capillaries is about two to one. The same study shows that when the muscle is worked open capillaries are present in nearly all of the fasciculi and the number of open capillaries per fasciculus is about doubled. Consequently the ratio of fibers to open capillaries becomes about three to four, the muscle as a whole showing about 3.3 times as many open capillaries as during rest. Furthermore, studies by Miriam Miller (unpublished), of the size of individual capillaries in worked as compared with resting muscles, have indicated the average increase in diameter to be of the order of 15 per cent, corresponding to an average increase in cross section of about 32 per cent. The net effect of the interaction of the above factors is to increase the width of the capillary bed by about 4½-fold, and this we consider the probable maximum increase under the conditions of our experiments. As pointed out in the paper cited above, a further increase (presumably of humoral origin and amounting conceivably to an approximate doubling) may be possible through the introduction of positive capillo-dilatation, but this implies other conditions than were present in the experiments here under discussion.

The blood supply to a muscle can also be increased by an increase in velocity of the blood flow through individual capillaries. In the present series of experiments, however, no increase in velocity of capillary flow occurred. On the contrary the increased blood flow through the muscle as a whole was only about 2½ times the resting value (see table 3), considerably less than the potential increase due to widening of the capillary bed. In connection with this observation we would call attention to the fact that there is a certain latency in the attainment of the maximum width of capillary bed (Martin, Woolley and Miller, loc. cit.). The latency is not sufficiently pronounced to prevent a striking increase in rate of blood flow from being manifest at the very beginning of activity, but presumably does operate to delay somewhat the attainment of maximum flow. It follows that the maximum increase in rate in our experiments may be supposed to have been somewhat greater than the total increase, given above as 2½ times the resting rate. No data are available as to the amount of difference, but observations presented below make it appear unlikely that the difference was sufficient to make the maximum rate exceed the resting rate by as much as the 4½-fold suggested above as the maximum probable increase in width of capillary bed.

The delivery of oxygen to active fibers. Another factor providing an increase in the supply of oxygen to active fibers is a greater dissociation of oxyhemoglobin. In our experiments the average discharge of oxygen into

the worked muscles was 13.2 cc. per 100 ml. of blood, as compared with a mean discharge of 10.5 cc. per 100 ml. into the same muscles before excitation (table 3), an increase of approximately one-third. Combining this with the maximum increase in width of the capillary bed estimated above we have a potential maximum increase in delivery of oxygen to individual fibers during activity amounting to nearly six-fold.

Other investigators (Verzar, 1912; Himwich and associates, 1927 and 1929) have reported a utilization of oxygen per 100 ml. of blood by active muscles of the same order as we have observed, but a considerably less utilization by muscles at rest. Consequently their data indicate a larger proportionate increase in oxygen delivery due to increased oxyhemoglobin dissociation; an approximate doubling, in fact. If their data on oxyhemoglobin dissociation are combined with ours on widening of the capillary bed a potential increase in oxygen delivery of about nine-fold is indicated. This figure seems to us significant in that it suggests the limit that will presumably be encountered in experimental studies of muscular metabolism of the type here under discussion. (The above limit was approached in the results reported by Himwich and associates, 1927, 1929; much less nearly in the work of Verzàr, 1912, of Barcroft and Kato, 1916, and in the experiments of the present series.)

Oxygen deficiency. At the moment stimulation is begun the muscle is in the resting state, receiving the blood-flow characteristic of that state and consuming oxygen at the resting rate, which in our experiments averaged 0.02 cc. O_2 per 100 grams muscle for the time occupied by a single contraction. The first contraction is always so nearly the maximum for the series that for the purposes of the present discussion it may be considered wholly so (see accompanying paper). An average total theoretical oxygen requirement of some 0.41 cc. is therefore established in each 100 grams of muscle with no immediate provision for meeting it beyond the limited provision afforded by an increase in the dissociation of oxyhemoglobin. In our experiments this factor increased the oxygen supply by only about one-third, hence before widening of the capillary bed begins to become effective the total available oxygen will not exceed 0.03 cc. per 100 grams of muscle to meet a theoretical requirement of about 0.41 cc. Coincident with the widening of the capillary bed the amount of available oxygen increases, reaching a maximum probably fairly early in the work period. Since in our experiments the average oxygen consumption for the entire work period was approximately three times the resting consumption (table 3) and the rate of consumption (parallel in general with the rate of blood flow) rose rapidly during the early stages of the period, the value of the maximum could not have been greatly in excess of the average. We postulate it, for purposes of illustration, at four times the resting figure. During the early part of an average series of 65 contractions the average oxygen utilization

per 100 grams of muscle per contraction is conceived, therefore, to have risen from about 0.03 cc. to about 0.08 cc. The theoretical oxygen requirement, on the other hand, is estimated to have declined by the end of the period from 0.41 cc. per contraction to a final value of the order of 0.10 cc. per contraction, the latter figure being computed from the average tension developed during a typical "steady state" (see accompanying paper).

The theoretical oxygen requirement *not met by oxygen* declines, according to the above calculations, from about 0.38 cc. per 100 grams of muscle per contraction at the beginning of exercise to about 0.02 cc. at the onset of the steady state. This part of the requirement is actually met by excess production of lactic acid. The equivalent quantities of acid for the above maximum and minimum are about 2.7 mgm. and 0.14 mgm. respectively.

A significant conclusion implied in the calculations given above is that whenever a skeletal muscle as a whole or *any group of fibers within it* contracts tetanically in the body a theoretical oxygen requirement is incurred which considerably exceeds the potential delivery of oxygen to the contracting fibers by the blood. This conclusion appears unavoidable in view of the maximal character of all contractions of individual fibers (under usual conditions of stimulation) considered in connection with the arrangement of the capillaries within the muscle, which is such as to localize sharply the potential sources of oxygen for individual fibers.

A second conclusion is that the greatest oxygen deficiency is necessarily incurred at or near the beginning of activity. This conclusion follows in part from the lag in widening of the capillary bed which has the effect of affording the muscle fibers a better oxygen supply after the exercise is well under way than at its very beginning, and in part from the reduction in force of contraction, and hence in quantity of phosphocreatine to decompose as activity continues. The above conclusions imply that when the contractions follow one another in quick succession, as in our experiments, the oxygen deficit mounts rapidly at first. We also have evidence that even though the production of lactic acid may not be rapid enough to meet the deficit as incurred the lactate concentration of the muscle also mounts rapidly. We have reported elsewhere experiments in which high concentrations of muscle lactate were found to be present after 30 seconds of rhythmic excitation (Martin, Field and Hall, 1930b), and we have seen in still briefer work periods, of 10 and 15 seconds respectively, increases in lactic acid concentration of 28 and 42 mgm. per cent, corresponding to a production per contraction of about 3.4 mgm. per cent. This figure corresponds nearly enough with the calculated lactic acid production attributable to the maximum contraction of an average work series, given above as 2.7 mgm. per cent, to lend support to the view stated on page 492 that the delay in acid production in mammalian muscles is less prolonged than in frog's muscles.

Fatigue. The conception that the force of contraction is determined (in general) by the amount of decomposition of phosphocreatine (p. 490) implies the further conception that decline in force of contraction, i.e., fatigue, is the result of a falling off in the amount of the same decomposition. Any factor which might operate to induce such a falling off thus becomes a potential cause of fatigue. The lactic acid theory of fatigue must obviously be tested from this point of view, as well as any alternative theories which suggest themselves.

One obvious alternative theory is that fatigue is due to accumulation of the decomposition products of phosphocreatine itself, an accumulation which may be conceived to reduce the volume of subsequent decompositions through a mass effect. The observation previously cited (p. 491) indicating a definite lag in resynthesis of phosphocreatine appears at first view to lend support to this idea, but the following considerations seem to us to throw grave doubt on its acceptability. According to the current picture of muscle metabolism, stimulating a muscle by a brief tetanus brings about an immediate explosive decomposition of some definite quantity of phosphocreatine, followed promptly by an onset of restorative processes, which may consist partly of reactions in which oxygen is utilized and partly of reactions in which production of lactic acid is the significant feature. The latter reactions, in turn, follow a course in which there is immediate production of a considerable fraction of the total amount of acid to be formed (in some cases not more than half; Hill, 1932, p. 63), followed by a declining acid production somewhat long drawn out, giving in the end, however, an energy-magnitude for the restorative process as a whole agreeing reasonably closely with the energy cost of resynthesizing the quantity of phosphocreatinine decomposed.

We cannot conceive how such a process as the explosive decomposition of a definite amount of an unstable compound from a considerable store of the same present in the muscle can of itself determine the magnitude and course of a subsequent restorative process such as we have under consideration here, but we can readily imagine that the immediate chemical result of the decomposition, namely, the accretion of a definite quantity of decomposition products, may so act. Proceeding on the latter basis we conceive, as the result of the sudden formation of some definite quantity of decomposition products of phosphocreatine, a definite series of reactions to be set in motion which almost immediately cause the recombination of a considerable portion into the parent compound, but leave a moiety to be recombined more gradually through the agency of that part of the lactic acid production which lags behind the rest.

Assuming a second brief tetanic stimulus to be applied shortly after the first the same sequence of events as described above is set in motion, but with the consequence that upon decomposition of the new quantity of phos-

phocreatine there is present a larger total quantity of decomposition products than at the corresponding stage following the first stimulus. Additional stimuli applied at the proper intervals accentuate the effect, giving thus the familiar phenomenon of summation. Our present interest is in the effect of this summation, not on force of contraction, but on the ensuing restorative processes. If we grant that the action of decomposition products in initiating and governing these restorative processes is susceptible of summation the processes themselves must also be thought of as summating in proportion. But the action of these restorative processes is to induce recombination of the decomposition products into phosphocreatine, hence to the extent to which they undergo summation they counteract the accumulation of the decomposition products themselves. As a matter of fact, as in other types of summation, a maximum will quickly be reached beyond which accumulation cannot continue. This is the significant point in our argument, for under any distribution of lactic acid production between the immediate phase and the delayed phase at all likely to occur in muscle, the maximum accumulation of decomposition products of phosphocreatine will be reached before fatigue phenomena begin to show themselves except perhaps to a very moderate extent.

Accumulation of lactic acid, on the other hand, will be particularly rapid during the period of summation and will continue thereafter as long as the energy-magnitude of the whole restorative process exceeds that of the oxidative phase, the only counteracting factor being the escape of lactates by diffusion into the blood. Our observation that lactic acid concentrations rise rapidly during the early stages of work periods (p. 496) is in harmony with this part of the conception. A contrary view, according to which decomposition products of phosphocreatine are conceived to accumulate, implies a correspondingly slower accretion of lactic acid, and is correspondingly less in harmony with the observed facts.

We conclude that the present trend of evidence, both our own and that offered by other investigators, tends to support the lactic acid theory of fatigue. We wish to make the point, however, that according to our observations the decline in developed tension which is interpreted as a manifestation of fatigue does not follow strictly the accumulation of lactates within the muscle. During the period of most rapid accumulation the tension developed in successive contractions remains nearly constant in many experiments. The suggestion is that there is some critical (and considerable) concentration of lactates which the fibers can endure without affecting the amount of phosphocreatine decomposition, but that after this critical concentration is exceeded further increases in concentration are accompanied by proportional or approximately proportional declines in the magnitude of the decomposition, and hence in the force of contraction.

The steady state. The above conception of fatigue leads directly to a con-

ception of the steady state as manifested in mammalian muscles in situ, according to which the state depends on the interaction of the following factors: 1, the decline in theoretical oxygen requirement as the force of contractions falls off; 2, the maintenance of the oxygen supply at its maximum, since the blood flow does not diminish; 3, the consequent decrease in production of excess lactic acid to a quantity which, at the diffusion gradient set up by the high concentration of lactic acid accumulated within the muscle, escapes by diffusion as fast as formed. When this situation is realized a metabolic equilibrium is established; no further accumulation of lactic acid takes place, and although no recovery is possible while the activity continues, neither is there a further decline in activity. It should be emphasized that this conception of the steady state implies it to be one of partial fatigue in which the active fibers are quite heavily charged with lactic acid.

Consequences of reduction in efficiency. The discussion thus far has proceeded on the assumption that in a given work period the tension developed in any contraction bears a fixed ratio to the quantity of phosphocreatine decomposed in the same contraction, although the possibility that under certain conditions the ratio may rise as fatigue advances is pointed out (p. 490). The tacit assumption has also been made that the energy cost of resynthesizing phosphocreatine is constant, although Meyerhof (1921) has shown that the "isometric coefficient of lactic acid," in other words, the ratio of phosphocreatine decomposition (as expressed in tension development) to the energy-magnitude of the restorative processes (expressed as lactic acid), falls considerably when fatigue becomes marked. These changes in ratio are in opposite directions and may conceivably counteract each other more or less completely in most instances. But we have some observations that seem to us to indicate that in certain cases the increased metabolic cost of resynthesizing phosphocreatine (a lowered isometric coefficient of lactic acid) considerably outweighs whatever increased economy of tension development may occur.

Whenever a muscle, under the conditions of our experiments, attains a steady state it does so, according to our interpretation, because the escape of lactates by diffusion moment by moment becomes equal to their production in the same intervals. This equality will obviously be reached sooner in a series of contractions in which the production of lactic acid decreases in strict proportion to the decline in tension development than in a similar series in which the production of acid per unit of developed tension rises. Even in the latter case, a steady state may be attained provided the change in the lactic acid coefficient is not too pronounced, but the level of tension development will obviously be lower. Or it may be that more lactic acid will come to be formed per unit of developed tension than can escape by diffusion at any stage, in which event lactates will continue to accumulate

until the muscle becomes wholly unable to contract. In the accompanying paper work periods are described which suggest the incidence of each of the three above described alternatives. All three are explicable in the main in accordance with our main conclusions, but by adding to the factors considered initially the further factor of fall in the lactic acid coefficient we arrive at an explanation of the phenomenon of muscular exhaustion, which is otherwise difficult to account for in the intact muscle in situ.

Consequences of partial activity. When the performance of the muscle is cut down as the result of the dropping out of the individual fibers in toto, either through myoneural junction "fatigue" or through failure of excitation of entire motor units, a somewhat different situation from that pictured thus far is presented. The inactive fibers are favored with the increased blood supply characteristic of the active muscle as a whole, but have a markedly reduced oxygen requirement, which must return nearly or quite to the basic level (some excess oxygen utilization may occur in metabolising the accumulated lactic acid). If the quiescent fibers are interspersed among active fibers the available oxygen supply to the latter is improved, their production of lactic acid is reduced to correspond, and the onset of fatigue is delayed in proportion. A steady state will presumably be established in course of time, but less rapidly in harmony with the less rapid onset of fatigue.

When the inactive fibers are in blocks, as is conceivably the case when entire motor units go out of action, or in the more pronounced situation in which the excitation is submaximal, leaving a considerable fraction of the muscle inactive, there will presumably be an increase in blood flow through the capillaries contiguous to these inactive fibers but too distant from active fibers for oxygen to penetrate from these vessels to them. The blood emerging from such capillaries into the veins must necessarily be less venous than the blood from the same vessels prior to the beginning of activity. Whether the effect can ever be so pronounced as to make the venous outflow from the muscle as a whole contain more oxygen during the work than prior to it is questionable. In the classical experiments of Chauveau and Kaufmann (1887) the blood from the resting muscle was invariably brighter than from the muscle in activity.

The metabolism of a muscle mass. With the above picture of the activity of individual muscles before us we turn to a consideration of the behavior of a large muscle mass exemplified in the experiments of our third group. As pointed out earlier (p. 490) the excess oxygen consumption per 100 grams of worked muscle per minute, calculated from the increased oxygen intake of the whole dog (table 5) is considerably larger than the corresponding figure determined directly on individual muscles in situ (4.7 cc. per 100 grams per minute during the first 3 minutes, as compared with about 1.8 cc. per minute during the 1.3 minute of the average experiment on single muscles).

This larger utilization of oxygen implies either a more copious blood flow or a greater arterio-venous oxygen difference. The weight of evidence indicates clearly, as already pointed out (p. 495), that the arterio-venous oxygen difference is not likely to be much greater than observed by us in these experiments, namely, about 13.5 volumes per cent. If we adopt this figure for the purpose of calculation, a blood flow of 42 ml. per 100 grams of muscle per minute must be postulated to permit of the utilization of 68 cc. of oxygen by 1200 grams of muscle per minute (4.7 cc. per 100 grams, plus 1 cc. per 100 grams basic oxygen consumption[3]). This is approximately twice the average flow through the worked gracilis muscle as given in table 3, but does not surpass the potential flow through a capillary bed widened to the maximum.

If we assume the blood flow to have been 42 ml. per 100 grams of muscle per minute the production and output of lactic acid in the experiments of this group can be estimated. The necessary additional data are given in table 4. They are strictly applicable only on the assumption that the diffusion of lactic acid from the active muscles into the venous blood is uniform moment by moment. But since the maximal production of acid occurs at the outset of activity, causing the muscles to become heavily charged with acid rather promptly (p. 496) the assumption has a sufficient degree of probability.

Forty-two milliliters of blood are thus presumed to have flowed through each 100 grams of active muscle per minute. According to the data of table 4 the average uptake of lactic acid by this quantity of blood was about 11 mgm. more than from resting muscle. In a work period of 15 minutes' duration each 100 grams of muscle is estimated, therefore, to have given off about 165 mgm. of lactic acid on the average. At the end of the work period each 100 grams of muscle still contained about 80 mgm. of lactic acid in excess of the quantity present in the resting muscle, indicating an average production of about 245 mgm. per 100 grams of muscle for the entire period of activity. It is clearly not legitimate to apportion this production of acid uniformly throughout the work period, since evidence has been presented painstakingly above to show that, on the contrary, the production is at its peak during the earliest stages of activity and declines thereafter to a considerably lower level. It happens that the average production of lactic acid per 100 grams of muscle per minute during work in the experiments summarized in table 3, agrees closely with the above figure for the excess acid remaining in the muscles of this series at the end of 15 minutes of activity, i.e., 80 mgm. If the amount estimated to have been taken up by the blood per minute, 11 mgm., be added to the above figure and the sum, 91 mgm., be presumed to represent the production of lactic

[3] This basic figure is taken from table 3.

acid per 100 grams of muscle per minute during the first stages of activity the following metabolic data and estimates are in hand:

Excess oxygen consumption per 100 grams of active muscle per minute 4.7 cc.; per contraction 0.094 cc.; basic oxygen consumption per contraction 0.02 cc.; lactic acid production per 100 grams per minute 91 mgm.; per contraction 1.8 mgm.; oxygen equivalent of 1.8 mgm. lactic acid, 0.250 cc.; total average theoretical oxygen consumption per contraction 0.346 cc. This estimate exceeds the mean theoretical requirement as determined more precisely on single muscle (p. 493) by about 20 per cent, but in view of the uncertain character of some of the factors, notably the arterio-venous oxygen difference, which was not determined directly in these experiments, the average weight of muscle stimulated, which was computed from muscle weighings in only one dog, and the lactic acid production of the whole leg musculature, which was assumed to be at the same rate as in the gracilis, which was the only muscle analyzed, the agreement is probably as close as can be hoped for, and seems definitely significant as supporting in considerable measure the conclusions based on metabolic data from single muscles as presented in antecedent paragraphs.

SUMMARY

1. The partition coefficient for lactates between muscles and blood of dogs is found to be 2.

2. The assumption that in unfatigued muscle tension development is a reliable index of phosphocreatine decomposition is discussed and accepted as valid.

3. The resynthesis of phosphocreatine through the combined agency of oxygen utilization and lactic acid production is considered.

4. The conclusion is drawn that in mammalian muscles in typical work periods the delayed lactic acid production is so nearly completed during the periods themselves that immediately subsequent determinations of muscle lactates include the whole theoretical amount, both immediate and delayed.

5. The average energy requirement, expressed as the "total theoretical oxygen requirement" per 100 grams of dog's gracilis muscle per brief isometric tetanus is calculated from determinations of oxygen consumption and lactic acid production during rest and activity.

6. On account of limitations imposed by the capillary circulation only part of the theoretical oxygen requirement of the individual active fibers in an active muscle is actually met by oxygen. The remainder is met by excess production of lactic acid.

7. The greatest oxygen deficiency, and hence the greatest production of lactic acid, necessarily occur at or near the beginning of activity. This follows in part from the lag in widening of the capillary bed at the beginning of activity, and in part from the reduction in theoretical oxygen requirement attending the reduction in force of contraction as activity proceeds.

8. Lactates necessarily accumulate rapidly during the early stages of activity, setting up high diffusion gradients for lactates between muscles and blood.

9. The work here presented is interpreted as supporting the lactic acid theory of muscular fatigue. An alternative theory, that fatigue is due to accumulation of decomposition products of phosphocreatine, is discussed and found doubtful. Accepting fatigue as due to accumulated lactates the fact is brought out that fatigue does not develop in strict harmony with the accumulation of lactates within the muscle but becomes manifest only after a considerable concentration is present.

10. The "steady state" is considered to be dependent on the interaction of the following factors: *a*, the decline in theoretical oxygen requirement as the force of contraction lessens; *b*, the maintenance of the oxygen supply at its maximum, since the blood flow does not diminish; *c*, the consequent decrease in production of excess lactic acid to a quantity which escapes by diffusion as fast as formed.

11. If the "efficiency" of the muscle falls off during the later stages of a work period a steady state may still be established at a low level of tension development, provided the relative increase in lactic acid production does not cause the actual quantity formed to exceed the maximum that can escape by diffusion. Should the production exceed this maximum the contractile power necessarily declines to complete exhaustion.

12. In muscles active only in part the active fibers may obtain additional oxygen through the increased blood flow to adjacent inactive regions within the range of effective oxygen diffusion. Beyond this range the active fibers in a muscle contracting submaximally can derive no benefit from the increased blood flow through the muscle as a whole.

13. Data obtained from a muscle mass, consisting of all the main muscles of one hind leg, support the above conclusions based on studies of single muscles.

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THE EFFECT ON OVULATION AND PREGNANCY OF BLOCKING THE PITUITARY CIRCULATION IN THE RABBIT[1]

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Although Gley (1891) made attempts to ablate the pituitary in rabbits, the first complete removal of the hypophysis was probably performed by Fee and Parkes in 1929. Following partial decerebration they were able to ablate the gland and keep their animals alive for nearly 48 hours. From results obtained with this technique they stated that rabbits did not ovulate if the pituitary was removed within one hour after mating, and that ovulation did occur if the hypophysis remained intact for an hour or longer after mating.

Smith and White (1931) with an operative procedure which neither exposed nor injured the brain and which was followed by prolonged survival, secured ovulation in animals in which the pituitary was left intact for an hour and a quarter after mating, a somewhat shorter post-copulatory period followed by ovulation than was observed by Fee and Parkes. They were also able to confirm the findings of Deanesly, Fee and Parkes (1930) that corpus luteum formation takes place even though the pituitary is removed shortly after mating and consequently several hours before ovulation. They found, however, that although the corpus luteum appeared normal for the first two days of its development, the subsequent stages were atypical and that actual regression was evident after four days.

The procedure of hypophysectomy employed by Smith and White usually requires a minimum of an hour for the completion of the operation. It seemed desirable to shorten this time in order to secure more precise data concerning the actual time after mating necessary for the anterior pituitary to liberate enough hormone to cause ovulation, and at the same time not to subject the rabbits to decerebration so that they would remain healthy for long periods. The information secured by a new operative procedure in which this has been achieved and which has given additional information on the length of the post-copulatory period of secretion of the pituitary necessary for ovulation forms the first part of the paper.

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Fraenkel (1910), Hammond (1925), and others have shown that the ablation of the corpora lutea (or ovaries) terminates pregnancy in rabbits. The final proof that the corpus luteum hormone is essential for gestation in this form has been given by Corner and Allen. Since the development of the corpora lutea becomes atypical after two days following hypophysectomy (Smith and White, 1931), it seemed probable that the fully-formed bodies would also soon become non-functional in the absence of the pituitary secretion. Data were secured on this point by operating on pregnant rabbits. The results obtained form the second part of the paper.

OPERATIVE PROCEDURE. The technique of blocking the blood circulation of the pituitary involved all the preliminary steps used by Smith and White in ablating the gland. Their procedure of hypophysectomy will therefore be briefly described. The mouth is opened and the jaws firmly secured by a head clamp. The dorsal mucosa of the nose is exposed by an incision through the soft palate, the opening being enlarged by a small retractor. The ophthalmic veins lying in the nasal mucosa are compressed as they pass through the cavernous foramen (Krause, 1883). The capacious blood sinus in which the hypophysis lies is then packed with bone wax inserted through the foramen, the foramen enlarged with a dental reamer, and the pituitary capsule exposed. The capsule is then torn open and its contents removed with a cannula and negative pressure.

Since the pituitary is surrounded by the lake of blood contained in the cavernous sinus (more accurately the circular sinus formed by the cavernous sinuses and the connecting transverse sinuses) and is separated from the brain by a bony shelf and the diaphragma sellae, it seemed probable that if the sinus were completely occluded with bone wax, the pituitary would be somewhat compressed and would suffer from an interrupted blood supply without injury to the brain. The procedure is not time consuming and can be completed without interference with the pituitary up to the stage of injecting the wax and then at the precise time desired the blockage be effected. That the animals suffer no brain injury from this technique is indicated by their healthy condition and their long survival without nervous disorders. A few animals, however, did develop symptoms approximately a month after the operation and at autopsy it was found that a purulent exudate had filled the sella turcica, raising the diaphragma sellae and thus causing pressure on the brain. These infections were infrequent but could not be entirely prevented.

For control operations all the steps were complete up to the injection of the wax.

The effect on ovulation. The data on the effect of this procedure on ovulation are given in table 1. The pituitary circulation was blocked at various periods between 30 and 90 minutes after copulation and exploratory laparotomies performed 24 hours later. None of the four animals

operated on earlier than one hour ovulated. Only one of three ovulated when the procedure was completed one hour after mating. The two animals operated on one and one-quarter and one and one-half hours, respectively, after mating both ovulated, results which confirm the earlier findings.

In the control operations (table 1) in which the mucosa was removed from the edges of the foramen as in the other animals, but no wax packed into the sinus, the normal percentage of animals ovulated, even when the procedure was completed as early as twenty minutes after mating. This shows that an incision through the oral mucosa and the pressure applied to the nasal mucosa to occlude the veins do not affect ovulation. The only additional trauma suffered by the experimental animals is limited to the

TABLE 1

Data on ovulation

ANIMAL	TIME AFTER MATING	OPERATION	REMARKS
	hours		
R42	20	Control operation	6 ruptured follicles
R39	25	Control operation	No ovulation
R40	30	Control operation	8 ruptured follicles
R41	30	Control operation	7 ruptured follicles
R50	30	Cavernous sinus occluded	No ovulation
R27	35	Cavernous sinus occluded	No ovulation
R28	45	Cavernous sinus occluded	No ovulation
R36	45	Cavernous sinus occluded	No ovulation
R29	60	Cavernous sinus occluded	No ovulation
R37	60	Cavernous sinus occluded	7 ruptured follicles
R38	60	Cavernous sinus occluded	No ovulation
R30	75	Cavernous sinus occluded	6 ruptured follicles
R31	90	Cavernous sinus occluded	5 ruptured follicles

contents of the cavernous sinus which makes it highly probable that the pituitary, only, is injured. The data presented in the first table therefore demonstrate that the pituitary must liberate its secretions for at least a full hour after mating to cause ovulation in the rabbit.[3]

The effect on pregnancy. The certainty that the complete occlusion of the cavernous sinus prevents the liberation of the pituitary secretion and the small amount of trauma incidental to the occlusion makes the opera-

[3] Since these experiments were completed two rabbits have been hypophysectomized within 50 minutes after copulation and one within 60 minutes. All three failed to ovulate. It has been previously shown that ovulation occurs if the pituitary remains intact for an hour and a quarter after mating. These data with the above experiments place the time necessary after copulation for the pituitary to remain intact to cause ovulation between 60 and 75 minutes.

tion of great value in investigations on the essentiality of the pituitary for the continuation of pregnancy. Fifteen pregnant animals were used, three for control procedures, seven for the occlusion of the sinus and five for the ablation of the pituitary. No operations were performed earlier than the seventeenth day of pregnancy since the fetuses are not readily palpable until about this time. The results are given in table 2. Abortion occurred in all of the experimental animals up to the 26th day of pregnancy, with the exception of R23 and R48, which died with badly macerated fetuses. Those operated on at the 26th and 28th days of pregnancy gave birth to both living and dead young. These deliveries

TABLE 2

Data on pregnant rabbits

ANIMAL	DAYS PREGNANT AT OPERATION	OPERATION	REMARKS
R32	17	Ether 2 hours	Normal pregnancy
R47	17	Control operation	Normal pregnancy
R51	25	Control operation	Normal pregnancy
R48	17	Cavernous sinus occluded	Died on 4th day, retained fetuses
R26	17	Cavernous sinus occluded	Aborted on 3rd day
R43	25	Cavernous sinus occluded	Aborted on 3rd day
R45	26	Cavernous sinus occluded	3 live young born on 4th day
R49	26	Cavernous sinus occluded	Aborted on 2nd day
R44	28	Cavernous sinus occluded	6 living young born on 3rd day
R35	28	Cavernous sinus occluded	5 live and 3 dead young born on 2nd day
R25	17	Hypophysectomized	Aborted on 3rd and 4th days
R20	21	Hypophysectomized	Aborted on 2nd day
R23	24	Hypophysectomized	Died 3 days later, macerated fetuses
R22	25	Hypophysectomized*	Aborted on 3rd day
R64	27	Hypophysectomized	Aborted on 3rd day, fetuses dead

* Small fragment of pituitary found in this animal at autopsy.

can possibly be considered abortions, however, since in no case were the young suckled or otherwise cared for.

From these findings it may be stated that the termination of pregnancy is a constant result of blocking the pituitary circulation. That this is due to a pituitary failure seems highly probable since pregnancy was not terminated in the majority of the animals until approximately 72 hours after the operation. If trauma played a significant rôle, abortion within 24 to 48 hours might be expected. That blocking the pituitary circulation produces the same effect as ablating the gland is shown by the five hypophysectomized animals. The most interesting of these animals is R64

which gave birth to non-macerated fetuses on the 30th day of pregnancy, 3 days after total hypophysectomy, indicating that the posterior lobe is not essential for initiating uterine contractions in parturition. The constant results from both procedures indicates the profound effect on the pituitary caused by the complete occlusion of the sinus.

All of the animals subjected to the control procedures gave birth to normal litters which were suckled.

HISTOLOGICAL CHANGES AFTER BLOCKING THE PITUITARY CIRCULATION. In part of the cases in which the cavernous sinus was occluded there was a complete necrosis of the entire pituitary with the exception of the stalk. The stalk, however, is not injured when complete hypophysectomy, as the term is generally applied, is performed. In other cases there was a fragment of normal hypophysis present. In animals with total destruction of the gland the histological findings were very similar to those described by Aschner (1912), Cushing (1912) and Ascoli and Legnani (1912), and others in the dog, and by Smith (1930) in the rat. Only very small follicles were present in the ovary. The uterus was of a castrate type and the vaginal mucosa was flattened. The zona reticularis of the adrenal had shown a fatty degeneration and the whole cortex was much diminished in width. The thyroids were subnormal in weight, and the follicular epithelium was much flatter than in normal animals. In animals with remnants of pituitary present, the histological picture was intermediate between the normal and those with complete degeneration of the gland. It therefore seems highly probable that the extent of the atrophy of the gland depends on the amount of wax packed into the sinus, which in turn determines the time necessary for a collateral circulation to be established. When the wax is packed in very firmly the ingrowth of new blood vessels is delayed until after the gland has become necrotic, while if the wax loosely fills the sinus it is possible for blood vessels to be reëstablished before autolysis of the glandular tissue has taken place. For these reasons this technic is unsuited for experiments which last for some time or for those in which a restorative therapy is undertaken. For an experiment of this kind hypophysectomy is superior. However, for studies in which a rapid operation entailing a minimum of trauma is desirable and in which the pituitary needs not be permanently blocked out, the technic presented here is highly satisfactory.

DISCUSSION. Although Fee and Parkes (1929) stated that the pituitary must secrete for one hour following mating, in order for ovulation to take place in the rabbit, they gave no data in their results for the interval between 32 minutes and 1 hour and 30 minutes after copulation. One of the purposes of the present investigation was to determine more exactly the actual time required by the anterior pituitary to liberate a quantity of hormone sufficient to cause ovulation. Our results confirm the state-

ment of Fee and Parkes since only one of three animals ovulated when the pituitary was blocked 60 minutes after mating and none ovulated when the operation was performed earlier. Ovulation was not interfered with if the gland was left intact for a period longer than one hour.

Aschner (1912) reported that hypophysectomy resulted in abortion in dogs. Smith and White (1931) reported abortion in a 22 day pregnant rabbit, three days after hypophysectomy. Pencharz and Long (1931) working on rats found that the hypophysectomy on the 11th to 20th day of pregnancy resulted in a prolonged gestation followed by death of the mother. Smith (1932) demonstrated that the posterior lobe is not essential for fertilization or parturition in this species. In view of the work of Jones (1926) it appears not improbable that the failure of the rats of Pencharz and Long to undergo parturition is due to the decrease in the tonicity of the uterus. That such is the case also appears probable from the work of the latter in which they found that parturition occurred if a fragment of the anterior lobe remained in situ. Allan and Wiles (1932) have recently reported abortion in cats after hypophysectomy.

In rabbits pregnancy is terminated after hypophysectomy and after blocking the pituitary circulation. That this is due to a loss of the pituitary hormones and not to trauma is demonstrated by the control operations. Furthermore, since the work of Smith and White has shown that after the ablation of the pituitary the corpora lutea develop normally only for a short period and since the corpus luteum hormone is essential for the maintenance of pregnancy in the rabbit, it seems probable that abortion is induced by hypophysectomy indirectly through failure of the corpora lutea. The final proof, however, that such is the case will depend upon the maintenance of pregnancy after hypophysectomy by the injection of corpus luteum extract. That abortion in the animals with blocked pituitaries is not due to an excess secretion of the posterior lobe is demonstrated by the fact that the removal of both lobes of the pituitary terminates pregnancy.

The writer wishes to express his appreciation to Dr. P. E. Smith for his assistance in this study.

SUMMARY AND CONCLUSIONS

The time required for the anterior pituitary to liberate the minimum quantity of hormone necessary to induce ovulation in the rabbit after copulation is between one and one and a quarter hours.

The blocking of the pituitary circulation results in the termination of pregnancy in the rabbit. Identical results are produced by ablating the gland.

The technique followed in blocking the pituitary circulation is given and the histological changes in the various organs following this procedure are briefly described.

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STUDIES IN ANAPHYLAXIS

I. The Appearance of a Physiologically Active Substance during Anaphylactic Shock[1]

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There is considerable evidence that the sudden, profound fall in arterial blood pressure that characterizes acute anaphylactic shock in the dog is mainly due to reactions taking place in the liver. This was indicated by the early work of Manwaring (1910a, b) and has subsequently been substantiated by the work of a number of investigators.

In this early work Manwaring reported that by cross circulating a normal dog with a dog rendered anaphylactic, anaphylactic symptoms were provoked in the normal animal. This led him to conclude that vasodilator substances are set free in the circulating blood as a part of the anaphylactic reaction. These positive experiments of Manwaring were contradicted by Weil (1917a) who failed to find any evidence of vasodilator substances in the blood of dogs dying from anaphylactic shock. He withdrew blood from the carotid arteries of dogs dying in anaphylactic shock and introduced this blood intravenously into normal dogs with negative results. Weil and Eggleston (1917) also reported negative results with blood repeatedly perfused through the isolated anaphylactic liver. Consequently Weil (1917b) was led to conclude, even though definite chemical changes in the blood of dogs during anaphylaxis had been shown by the work of Jobling, that "transfusion experiments have revealed the fact that these chemical changes are without influence upon the development of the shock. They are simply the harmless by-products of the anaphylactic reaction of the sensitized liver, and as a matter of fact, accompany a variety of pharmacologic procedures which injure the liver, such as chloroform or phosphorus poisoning."

In 1925 Manwaring and his co-workers (Manwaring, Hosepian, O'Neill and Moy), although corroborating Weil's experiments that the transfusion into normal dogs of carotid blood drawn from dogs dying in anaphylactic

[1] A preliminary report of this paper was made before the Illinois Branch of the Society for Experimental Biology and Medicine, April 5, 1932 (Proc. Soc. Exper. Biol. and Med., 1932, xxix, p. 891).

shock was without definite effect, and apparently admitting that the earlier cross circulation experiments were not conclusive, nevertheless reported definitely positive results by transfusing hepatic vein blood of anaphylactic dogs into normal dogs. They also reported that the vascular anastomosis of a normal dog's hind quarters to an anaphylactic dog demonstrated typical though slight anaphylactic contractions in the urinary bladder and rectal stump of the normal animal. To the physiologically active substance or substances indicated by these experiments to be set free in the blood stream they gave the name of hepatic anaphylatoxin.

These positive and apparently conclusive experiments of Manwaring have prompted us to investigate the appearance and possible nature of such hepatic anaphylatoxins. Inasmuch as Manwaring's observations have not been universally accepted, we were desirous first of either confirming or denying the reported appearance of physiologically active substances during shock. As an indicator of such activity we chose the guinea-pig intestine because the presumptive evidence is very strong that at least one of the properties of a physiologically active substance having either a cause or effect relationship to the symptoms of anaphylactic shock should be that of contracting smooth muscle. In addition to looking for such activity in the blood we were interested in examining the thoracic duct lymph, since Calvary (1911) and Petersen and Levinson (1923) have noted a marked increase in the amount as well as marked changes in composition of the lymph during anaphylactic shock.

The dogs were sensitized by a single intravenous injection of from 1 to 8 cc. of horse serum or hog serum. In a few instances a simultaneous injection of a similar amount of serum was made subcutaneously. In four cases sheep serum was used as the antigen but as well marked anaphylactic shock did not develop in any of the four, it was discontinued. The relative ineffectiveness of sheep serum as a sensitizing antigen in the dog has been noted by previous workers. After an interval of fourteen days or longer, the dogs were anesthetized, cannulae inserted in the carotid artery and thoracic duct for recording blood pressure and collecting lymph respectively. The shocking dose of serum was injected into the femoral vein. During the course of ensuing shock, lymph was collected in small beakers, blood was withdrawn by syringe from the femoral vein and in some experiments from the portal vein and the inferior vena cava. For the latter procedure, the chest was rapidly opened and the needle inserted into the inferior vena cava about 2 cm. above the diaphragm. Although this method yielded hepatic vein blood diluted to a varying degree with other blood, it was considered desirable to avoid the trauma incident to methods permitting the collection of exclusively hepatic venous blood. If the shock lymph or blood samples showed any evidence of clotting, additional samples were collected to which heparin was added. The collected samples

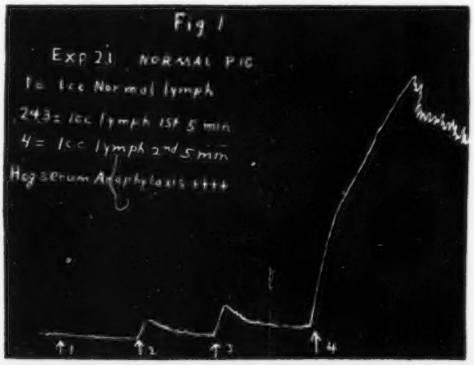
of lymph and blood were tested for physiological activity by means of a strip of guinea-pig intestine suspended in a 200 cc. bath of aerated Tyrode's solution at 39°C. in the usual way.

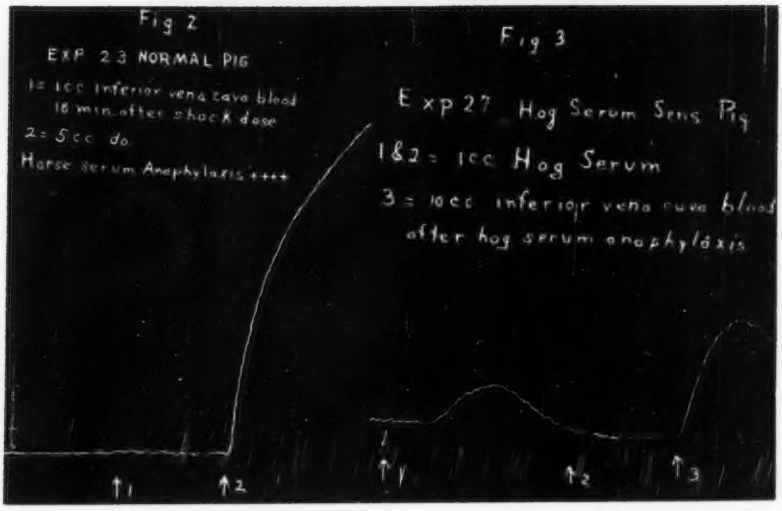
An illustrative protocol is as follows:

Experiment 23. Female dog, 10 kilos.
January 29, 1932. 4 cc. horse serum, saphenous vein.
February 19, 1932. 1:00 p.m. Sodium barbital 0.275 gram per kilo intravenous.
Cannula in carotid for blood pressure. Cannula in thoracic duct.
2:30. 10 cc. blood withdrawn femoral vein, heparin.
2:45–3:00 p.m. Collection of normal lymph, 8 cc.
3:00. 10 cc. horse serum femoral vein. Marked fall in blood pressure after latent period of 40 seconds.
3:00–3:05. First sample of "shock lymph," 8 cc.
3:05–3:10. Second sample of "shock lymph," 10 cc.
3:10–3:20. Third sample of "shock lymph," 10 cc.
3:18. 10 cc. blood withdrawn from femoral vein. Dog apparently dying.
Chest rapidly opened and 50 cc. blood aspirated from inferior vena cava.
3:20. Dog dead.
Blood and lymph samples immediately tested on guinea-pig intestine.
10 cc. normal femoral vein blood plus heparin, no effect.
8 cc. normal lymph, no effect.
1 cc. first sample "shock lymph," definite contraction.
1 cc. second sample "shock lymph," definite contraction.
1 cc. third sample "shock lymph," very marked contraction.
½ cc. third sample "shock lymph," definite contraction.
10 cc. shock femoral vein blood, no effect.
1 cc. shock vena cava blood, slight contraction.
2 cc. shock vena cava blood, definite contraction.
5 cc. shock vena cava blood, marked contraction.

Tracings illustrative of the contractions obtained are shown in figures 1, 2, and 3 with legends which are self-explanatory. Attention is called to the fact that the contraction occurs immediately upon the addition to the bath of the active specimen of lymph or blood. This is particularly illustrated in figure 3 in which the small intestine of a sensitized guinea pig was used for the testing. Here a typical anaphylactic contraction with the usual latent period was first induced, and then after the intestinal strip had been thus desensitized, an active specimen of inferior vena cava blood produced an immediate contraction. Normal blood, drawn from either normal dogs, or from the sensitized dogs prior to shock and tested immediately or kept from clotting by heparin was consistently inactive even in doses of 25 cc. Active specimens of inferior vena cava blood obtained after shock, although incoagulable, did not lose any activity by the addition of heparin. Many samples of normal lymph were tested prior to clotting and were consistently inactive. Those prevented from clotting by heparin were likewise inactive. The addition of heparin to active specimens of shock lymph did not diminish their activity.

Most of the essential details of the experimental methods and results are indicated in the table. Attention is called to the method of grading the severity of the anaphylactic shock. Fatal shock is graded ++++; non-fatal shock in which there is no appreciable recovery of the blood pressure from its low level within the period of one-half hour is graded





+++; non-fatal shock in which the blood pressure has been markedly reduced but tends to recover within one-half hour is graded ++; and shock in which there is a negligible fall in blood pressure is graded +.

Thus it is seen that of twenty-seven cases of anaphylactic shock of varying degree in which the thoracic duct lymph was tested for physiological

activity on the guinea-pig intestine, the lymph was definitely positive in ten, questionably positive in two and negative in fifteen. However, of the eleven cases of severe or fatal shock, the lymph was positive in eight. Similarly the inferior vena cava blood was positive in eight experiments

TABLE 1

EXPERIMENT	KILOS	SENSITIZING DOSE OF SERUM	INTERVAL	SHOCK DOSE	DEGREE OF SHOCK	SMOOTH MUSCLE STIMULATING PROPERTY OF				REMARKS
						Thoracic lymph	Inferior vena cava blood	Portal blood	Femoral blood	
		cc.	*days*	*cc.*		*cc.*	*cc.*	*cc.*	*cc.*	
3	8	1.25	21	3	++			−10	−10	
4	6.3	2	14	3	++++	+1				
5	6.3	2	15	3	++++	−5				Lymph tested after 48 hrs.
7	11	1½	15	3	++	+?5				
8	11	3	19	3	++	+?5	−10			
9	8.4	3	22	5	+++		+2			
10	7	3	23	5	+++	+1				
11	12.5	4.5	22	4	+	−5				
12	15	6	20	8	++++	+0.5				
13	12.7	5	27	5	++	−5				
14	16	5	20	7	++	−10				
15	19	8	22	7	+++	−10	−10			
16	10	5	30	10	++	−5				
17	17	5	16	10	++	−5				
18	12	3	16	10	++	+4				
19	11	3	19	7	++	−7	−10			
20	15.5	3	23	10	++	+0.5				
21	9.3	3	17	10	++++	+0.25	+4		−10	
22	11	3	25	10	++	−20				
23	10	4	21	10	++++	+0.25	+2		−10	
24	14	4	28	10	++++	+2	+5		−10	
25	19	4	21	10	+		−10		−10	
26	12	4	16	10	+	−5			−10	
27	12	4	21	10	++++	−6	+5		+10	
28	28	4	26	11	++	−10			−10	
31	11	4	45	11	++	−5				
35	17	3	25	10	++++	+1	+1	+10	−10	
36	11	3	27	10	++	−3	+5	−10		
37	26	4	36	10	++++	+5	−10	−20	−15	
38	15	3	18	10	++	−5	+25			

out of thirteen examined, while in fatal shock it was positive in five out of six. There is therefore some definite correlation between the severity of the shock and the appearance of the active substance. This correlation is not absolute as in the case of experiment 20, with a relatively mild degree

of shock, a highly active lymph was obtained, and in the case of experiment 27, with a fatal shock the lymph was inactive at least in doses of 6 cc. In most of the experiments in which both the inferior vena cava blood and thoracic duct lymph were examined there was a comparable degree of activity in both. However, in experiment 27 the blood was active and the lymph inactive (in doses of 6 cc.) while in experiment 37 the lymph was active and the blood inactive (in doses of 10 cc.). It is to be noted that the determination of physiological activity is expressed as positive or negative with respect to certain doses of lymph or blood added to the 200 cc. bath containing the intestinal strip, so that in some instances it is possible that a lymph or blood sample recorded negative in the specified amount might have been positive if further quantities had been available for testing. In every instance the irritability of the guinea pig intestine was checked by a standard solution of histamine. Therefore in most of the instances of recorded positive specimens of lymph or blood appearing in the table the accompanying quantity specified indicates that this amount provoked a contraction comparable to that produced by 0.001 mgm. of histamine dihydrochloride (e.g., in experiment 23, 0.25 cc. of lymph provoked a contraction equivalent to that of 0.001 mgm. histamine dihydrochloride and the activity of the lymph was approximately equivalent to that of a 1 to 250,000 solution of histamine dihydrochloride).

While not systematically studied in every experiment, considerable data were obtained with respect to the time relationship of the appearance of the active substance. Thus in several experiments in which adequate amounts of lymph were collected within three minutes from the time of injection of the shocking dose of serum to warrant testing, it was clearly evident that the activity was present at this early period. This was the case in all instances of fatal shock. In one case of non-fatal shock (++) the first five minute sample of thoracic duct lymph was negative in 3 cc. doses, while the second five minute sample was positive in 4 cc. doses. The time relationship was somewhat clearer in the case of experiment 12 (fatal shock). The first three minute sample was negative in 1 cc. doses, but positive in 5 cc., the sample collected between the third and sixth minute was positive in ½ cc. doses, the sample collected between the sixth and fourteenth minute was positive in 1 cc. doses, and the sample collected between the fourteenth and twentieth minute was positive in 2 cc. doses. Here a definite rise and fall in the concentration of the active substance is indicated. In one experiment (expt. 9) in which the chest was kept open and the animal under artificial respiration, the inferior vena cava blood was positive three minutes after the shocking dose in amounts of 2 cc. and negative in doses of 10 cc. ten minutes later. In one case of non-fatal shock (expt. 20) the thoracic lymph was positive in ½ to 1 cc. doses for thirty minutes after the shocking dose, then becoming negative in 4 to 10 cc. doses. It is apparent

therefore that the active substance appears in the thoracic duct lymph or inferior vena cava blood very promptly upon the onset of shock, and, if the shock is non-fatal, will decrease and disappear in a variable but comparatively short space of time.

Our experiments were not designed or performed to permit of any positive conclusions as to the source of the active substance appearing during shock. Our results are for the most part compatible with the conclusions of Manwaring that the chief source is the liver. This is suggested for example by the observations in experiment 37 in which it was shown that the activity is present in the inferior vena cava blood but not in the portal vein blood. Similarly, as the evidence is quite strong that the increase in thoracic duct lymph occurring during anaphylactic shock is chiefly of hepatic origin, there is a considerable degree of probability to the inference that the active substance appearing in such shock lymph comes from the liver. On the basis of our experiments alone, however, other possibilities would probably have to be considered.

The almost regular absence of activity in the femoral vein blood would seem to indicate that the active substance disappears rapidly from the blood as it circulates. This is in harmony with the observations of Manwaring and his co-workers who found that the transfusion of carotid blood from dogs dying in anaphylactic shock into normal dogs was essentially negative while the transfusion of hepatic vein blood produced definite anaphylactic symptoms. It is important to recognize, therefore, that this loss of activity on the part of the blood as it circulates may largely explain the negative results of numerous investigators who have failed to find physiologically active substances set free during anaphylactic shock.

During the course of the preceding discussion we have frequently referred to the blood or lymph specimens that had the property of stimulating the guinea-pig intestine as containing a physiologically active substance. That the acquisition of this activity by the blood or lymph is really due to an active substance is definitely shown by the experiments in the succeeding paper.

We do not propose to discuss at length what significance attaches to the appearance of a smooth muscle stimulating substance or substances in the inferior vena cava blood or thoracic duct lymph during anaphylactic shock in the dog. The proper interpretation will follow upon the elucidation of all the facts. Whether it represents a humoral toxic agent resulting from the anaphylactic reaction of antigen and antibody which serves to provoke some or all of the symptoms of anaphylactic shock or whether it is merely the harmless by-product of such a reaction is not demonstrated by these experiments. Or a third position may be taken, namely, that this active substance may indeed be identical with the toxic substance mediating some or all of the symptoms of shock, but that the amount which appears free

in the blood or lymph represents the excess production over and above that necessary to produce symptoms. As there is no distinct amelioration of shock produced by draining the thoracic duct lymph to the outside it is apparent that that portion of the active substance appearing in the lymph may be regarded as such an excess or at least a relatively unimportant fraction of that which is producing symptoms. It remains, however, to determine the nature and production of the active substance and the extent to which it may produce effects comparable to those appearing during shock.

CONCLUSIONS

1. In the majority of instances of severe or fatal anaphylactic shock in the dog there appears a substance or substances which have the property of stimulating smooth muscle (guinea-pig intestine) in *a*, the supra-diaphragmatic inferior vena cava blood, and in *b*, the thoracic duct lymph.

2. This substance apparently disappears or rapidly diminishes as the blood circulates as it can but rarely be demonstrated in the femoral vein blood.

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STUDIES IN ANAPHYLAXIS

II. The Nature of a Physiologically Active Substance Appearing during Anaphylactic Shock[1]

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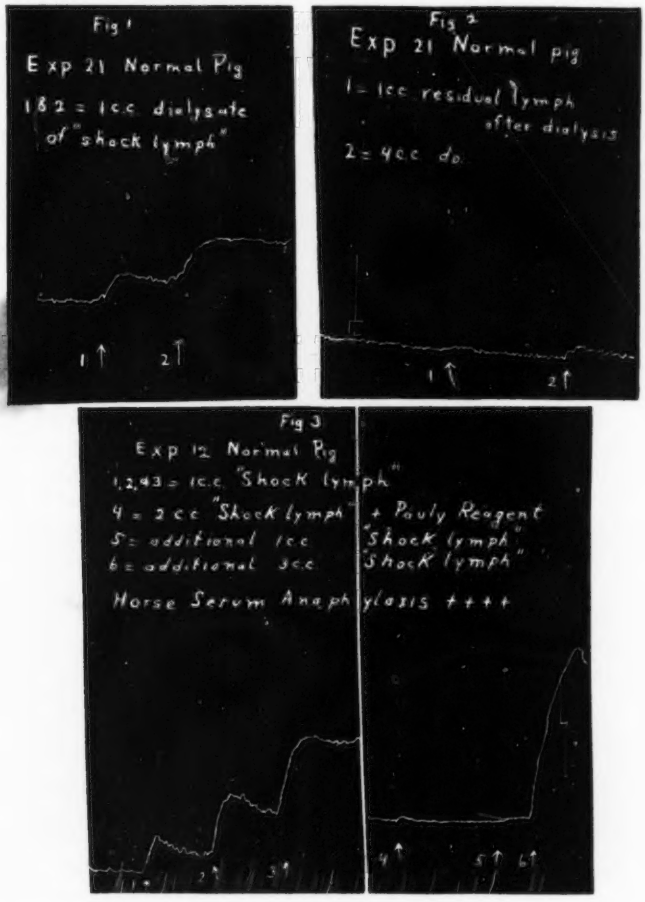
It has been shown in the preceding paper that a physiologically active material may appear in the inferior vena cava blood and thoracic duct lymph of dogs during anaphylactic shock. In order to characterize the principle encountered a number of chemical and pharmacological experiments have been performed and are here reported.

1. The active material is dialyzable. The first question that presented itself was as to the molecular size of the active material. Dialysis of active blood and lymph samples was resorted to. Parchment sacs as well as cellophane tubing were used as semi-permeable membranes and the experiments carried out by dialyzing into an equal volume of either distilled water or 0.85 per cent sodium chloride for ten to twelve hours in the ice-box. In some instances a few drops of toluene were added to prevent bacterial growth, although even when not added no apparent bacterial growth took place. Care was taken to start the dialysis and transfer the experiment to the ice-box immediately after collection of the blood or lymph sample. When distilled water was used the dialysate was concentrated in vacuo to a small volume and the final concentrate adjusted to the proper pH for further testing. When normal saline was used the dialysate was not concentrated. The dialysates of active samples of blood or lymph were invariably active in stimulating the guinea-pig intestine (see fig. 1). The blood or lymph samples after dialysis retained little or none of the previous activity (see fig. 2). This experiment demonstrated the fact that the active substance is a crystalloid of relatively small molecular size. Recent work by Coca (1930) indicates that proteins ordinarily thought to be non-diffusible will pass through semi-permeable membranes in small quantities during the course of four or five days. However, the ease with which our material diffused leaves little doubt as to its molecular size.

[1] A preliminary report of this paper was made April 30, 1932 at the meeting of the American Physiological Society.

2. The active substance is predominantly basic. Having observed that the active substance dialyzed through semi-permeable membranes, the next step was to ascertain what type of compound it represented, whether acidic, basic or amphoteric. For this means electrodialysis was employed.



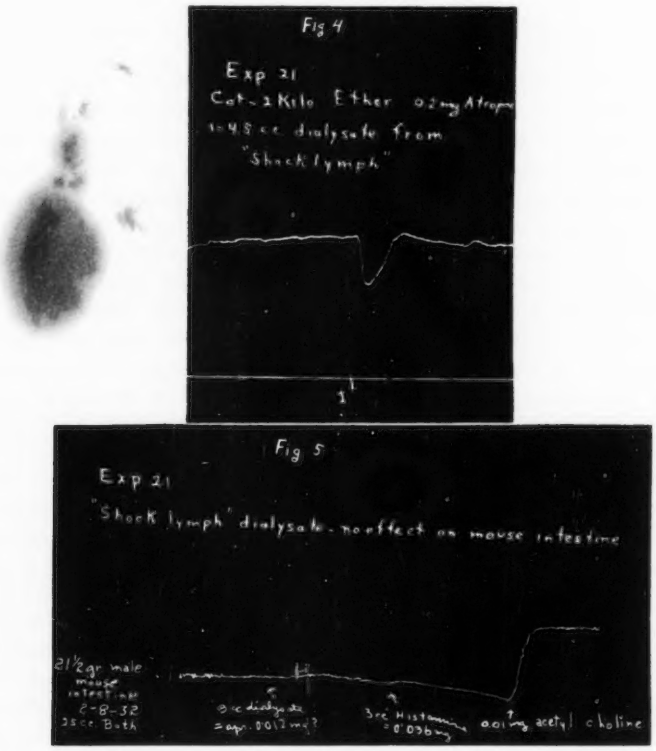
Active samples of shock blood or lymph were placed in the middle cell of a three-compartment electro-dialyzer. The two outer cells, fitted with platinum electrodes, were filled with distilled water or normal saline. Under an electric potential the activity of the middle cell rapidly diminished, while that of the basic cell increased correspondingly. The cathode

and anode compartments were cooled during the experiment. To prevent excess alkalinity which conceivably might destroy the active material, CO_2 was bubbled through the basic compartment. The middle cell was kept at approximately the original pH by appropriate additions of alkali. These experiments indicated that the physiologically active substance was either a base or predominantly basic in character at the pH of the electrodialysis. It has previously been shown (Gebauer-Fuelnegg and Kendall, 1931) that physiologically active bases can be separated from protein mixtures by electrodialysis.

3. The active substance is not specific. Since the active material has a small molecular weight we should not expect it to be immunologically specific. To confirm this view a series of guinea pigs were sensitized to and a second series immunized against hog or horse serum respectively. Active specimens of blood and lymph were obtained from dogs during anaphylactic shock induced by either hog or horse serum. Such active samples were equally active in stimulating the intestine of guinea pigs whether normal or sensitized to, or immunized against the corresponding serum. Similarly the blood or lymph from hog serum anaphylactic dogs was equally active when tested on horse serum sensitive or horse serum immune guinea pigs and vice versa the blood or lymph from horse serum anaphylactic dogs was equally active on hog serum sensitive or hog serum immune pigs. For the most part the immune guinea pigs had a titer of from 1 to 500 to 1 to 1000. As was pointed out in the preceding paper the contraction of the guinea pig intestinal strip induced by an active shock blood or lymph sample is an immediate one and does not have the latent period so characteristic of an anaphylactic contraction induced by the addition of the antigen to an intestinal strip of a sensitized animal. On the basis of these findings we conclude that the active substance is identical irrespective of the antigen used and definitely lacking in any specificity. This is contrary to the view expressed by Manwaring, Marino, McCleave and Boone (1927).

4. The active substance is inactivated by diazotized sulphanilic acid. In the course of the aforementioned experiments it was noticed that diazotized sulphanilic acid will inactivate the active substance in the presence of an excess of sodium carbonate. Solutions of the red condensation product (Pauly's reaction) failed to contract the guinea pig intestine after neutralization of the excess sodium carbonate. (See fig. 3.) We know that only a limited number of naturally occurring amino acids or amines will give condensation products with diazotized aromatic amines or their derivatives: tyrosine and tyramine as well as histidine and histamine. In addition protein split products of higher molecular weight such as peptides, etc., containing these amines or amino acids will also give a positive Pauly reaction. The inactivation by diazotized sulphanilic acid

seems a very important indication that the active material belongs to the group of compounds just mentioned. From this group we can exclude from consideration the two amino acids and probably all peptides except possibly those containing histamine or tyramine. With respect to histamine it has recently been shown (Gebauer-Fuelnegg, 1932) that under proper conditions two mols of diazotized sulphanilic acid will effectively inactivate one mol of the base. It is realized, however, that a physio-



logically active substance hitherto unknown might exist that gives a positive Pauly reaction.

5. The active substance causes a fall in blood pressure in the etherized, atropinized cat.

Since the reactions of the active substance reported above were in accord with the assumption that it may be histamine, a number of further tests were made that are more or less typical for this substance. A dialysate

from an active shock lymph, part of which was first assayed on the guinea-pig intestine was injected intravenously into an etherized and atropinized cat. A prompt, temporary fall in blood pressure resulted. See figure 4.

The assay of the dialysate on the guinea-pig intestine expressed as histamine equivalent was closely approximate with that of the assay on the cat when similarly expressed. Inasmuch as the depressor response in the cat is considered by many as definitely characteristic of histamine and there is good quantitative agreement between the two methods of biological assay on the assumption that the active substance is histamine considerable support to such an assumption is afforded by these experiments.

6. The active substance produces characteristic wheals. Dialysates of active lymph and blood samples were found to produce characteristic wheals when endermally inoculated in human skin. While this reaction is by no means specific, it is consistently found with histamine solutions of adequate concentrations. Control wheals were produced with histamine solutions of known concentration and the values obtained by the wheal test were in harmony with those by the preceding methods of assay.

7. The active substance does not contract the mouse intestine. It has recently been reported (Wedum and Gebauer-Fuelnegg, 1932) that the intestine of the mouse is insensitive to histamine while highly reactive to acetyl choline. Dialysates of active specimens of blood or lymph, highly active on the guinea pig intestine, were without effect on the mouse intestine. See figure 5. This, as do a number of the preceding experiments, definitely excludes acetyl choline from consideration.

8. Miscellaneous.

Active specimens of lymph or blood retain their activity for at least one week in the ice-box, but lose their potency if kept in the incubator for twenty-four hours. Dialysates of active lymph or blood, brought to pH 7.6, have their activity diminished by prolonged drying on the water bath. Slightly acidified dialysates are stable to boiling. Specimens of normal blood and lymph to which histamine was added in comparable amounts gave similar results when carried through the above procedures. The contraction of the guinea-pig intestine induced by an active specimen of blood or lymph or their dialysates, is relaxed by the addition of formaldehyde. It has been shown that this is also the case in contractions induced by histamine and the anaphylactic reaction. (Kendall and Varney, 1927.)

SUMMARY. Summarizing the evidence presented in the preceding sections, it may be stated that the active substance occurring in the inferior vena cava blood or thoracic duct lymph of dogs during anaphylactic shock, has the following characteristics: It is a dialyzable crystalloid of basic

properties. It stimulates the smooth muscle of the guinea-pig intestine obtained from pigs that are either normal or sensitive or immune to either the homologous or a heterologous serum. The activity is associated with substances giving the Pauly reaction as it is inactivated by condensation with diazotized sulphanilic acid. It depresses the blood pressure of the etherized, atropinized cat but does not contract the smooth muscle of the mouse intestine. It produces characteristic wheals in human skin. Acid solutions are stable to boiling, while neutral or slightly alkaline solutions are somewhat unstable to heat. All of the above criteria are in accord with the conclusion that the active substance is histamine. This conclusion is strengthened by the fact that if histamine in adequate concentration is added to normal blood or lymph samples and these then carried through the same procedure similar results are obtained. In addition there is quantitative agreement between histamine solutions and the active substance when tested by three widely different methods of biological assay.

Although the above evidence would seem to be conclusive, it would be desirable to isolate histamine in crystalline form or to include spectroscopic evidence of its presence. However, the quantities of histamine indicated by our studies to be present are not such as to warrant the hope that either method would be satisfactory. For example, the maximal amount of active lymph obtained in a single experiment was 50 cc. and the maximal amount of inferior vena cava blood 200 cc. Assuming the average activity to be 0.001 mgm. histamine dihydrochloride per cubic centimeter of lymph and 0.0005 mgm. per cubic centimeter of blood, we are dealing with a total of 0.15 mgm. dihydrochloride or approximately 0.09 mgm. of base.

In the experiments where more active samples of lymph or blood were obtained the volumes were generally smaller owing to the rapid death of the animal. Data soon to be published indicate that spectroscopic assays of histamine are not delicate enough to determine these small quantities. Pooling the samples of several experiments has not yet been done.

Experiments on the inactivation of the active substance by the specific enzyme histaminase described by Best and McHenry (1930) are now in progress.

As to the origin of the histamine here shown to be present in the inferior vena cava blood and thoracic duct lymph during anaphylactic shock, there are a number of possibilities to be considered. Among them may be mentioned 1, the possibility that histamine originates from the blood; 2, that it is of cellular origin; 3, that it comes from the gastro-intestinal tract, and 4, that it comes from the liver secondary to the engorgement resulting from the shock. A discussion of these points is contemplated for the near future.

CONCLUSION

The active substance shown in the preceding paper to appear in the inferior vena cava blood and thoracic duct lymph during anaphylactic shock in dogs is apparently histamine.

Upon the completion of this paper our attention was called to a paper by Bartosch, Feldberg and Nagel in Pflüger's Archives of May 24, 1932. These authors report in a very interesting paper perfusion experiments on the lungs of anaphylactic guinea pigs demonstrating the occurrence of a physiologically active substance in the perfusates. They concluded that this substance was histamine. This is a valuable confirmation of our experimental findings from an entirely different method of study.

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THE INTRAPERITONEAL ADMINISTRATION OF VIOSTEROL IN MICE

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The aim of this study was to determine if irradiated ergosterol[2] is efficacious when injected into the peritoneal cavity.

During the course of experiments dealing with the calcification of the Twort mouse carcinoma (in vivo) by means of viosterol (7), the animals receiving medication frequently had diarrhea. It was presumed that such an abnormal condition of the gastro-intestinal tract might have not only an adverse effect upon the health of the mice but might well lead to a non-utilization of much of the viosterol. Therefore, the present investigators were confronted with the finding of a parenteral route by which the activated ergosterol might act efficiently and yet cause no effects detrimental to the object of the investigation. The decision to inject the substance into the peritoneal cavity was influenced by the report of Koehne and Mendel (1) which stated that the Vitamin D principle in cod liver oil afforded some protection to rats when given in this manner, and our enthusiasm was kindled somewhat by the fact that (at that time) we found no articles dealing with this mode of administration for viosterol.

EXPERIMENTAL MATERIALS AND METHODS. The irradiated ergosterol, as received from the manufacturers, was concentrated in sesame oil to a biologically-assayed strength of 10,000 times the antirachitic potency of cod liver oil (10,000 CLO). In order to obtain solutions of 1,000 CLO and 2,000 CLO, which were used in certain phases of the present investigation,

[1] Submitted to the Faculty of the Yale School of Medicine as partial requirement for the degree of Doctor of Medicine.

[2] The viosterol was obtained through the courtesy of Mead Johnson & Co., Evansville, Indiana.

Such terms as viosterol, irradiated ergosterol, and activated ergosterol are used interchangeably throughout the present article.